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Department of Pharmacology

School of Medicine

University of California

- Los Angeles -

Contract No. DA 18 108 405 CML 735

FINAL REPORT

1 April 1960 - 31 December 1962

I. METHYLENEDIOXY-AMPHETAMINE HALLUCINOGENIC SERIES OF COMPOUNDS

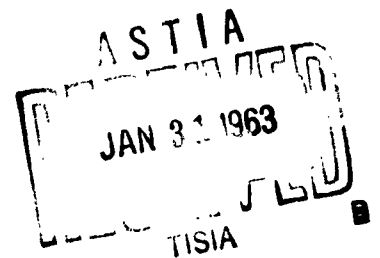II. CONDITIONED BEHAVIOR AND ELECTROENCEPHALOGRAPHIC TEST METHODS

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and

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December 1962

CONTRACT NO. DA 12 108 405 CML 735

FINAL REPC..T

4/1/60 - 12/31/62

I. METHYLENEDIOXY-AMPHETAMINE HALLUCINOGENIC SERIES OF COMPOUNDS

There was submitted about 10 grams each of the salts of four 3,4-methylenedioxy-amphetamine derivatives to the Directorate of Medical Research, Army Chemical Center. Preliminary animal work on toxicity and relative motor activity had been carried out on these compounds; further animal work and studies of their relative hallucinogenic activity in man was to be the responsibility of the Directorate of Medical Research. A short summary of some animal work (behavioral studies in cats) was presented to us by Dr. David McCurdy, our project officer, in July 1961 and no further report was received on these compounds from the Director of Medical Research.

It was hoped that another small, closely related series of compounds, whose potency for producing certain behavioral and electrophysiological changes in experimental animals has been investigated by us, would also have been valuated by the Army Chemical Center for their hallucinogenic activity in man. This goal failed of any notable achievement.

II. CONDITIONED BEHAVIOR AND ELECTROENCEPHALOGRAPHIC TEST METHODS

A behavioral study on the effects of a selected series of four substituted amphetamine derivatives on conditioned avoidance in the mouse was carried out. Mescaline and amphetamine were used as the comparison test substances.

The apparatus used and the results of our studies are detailed in the attached copy of the Ph.D. thesis of M. D. Fairchild.

In summary, the results obtained indicated that 3,4,5-trimethoxy-amphetamine (TMA) and 3,4-methylenedioxy-amphetamine (MDA) were the most potent amphetamine derivatives for disrupting avoidance behavior in the mouse. The compound 3,4-dimethoxy-amphetamine (DMA) was the least potent while 3,4-methylenedioxy-5-methoxy-amphetamine (MMDA) appeared to be intermediate between DMA and the more active MDA and TMA. Mescaline had a potency comparable to DMA while amphetamine itself ranked with MDA and TMA.

Prior to our entering into a contract with the Army Chemical Center to study the four substituted amphetamine derivatives, a pre-liminary study of their activity on transcallosal conduction in the cat, under the certain defined experimental conditions of Marrazzi and Hart, was carried out. These investigators considered such valuations to be related to the hallucinogenic activity of mescaline, LSD, and certain tryptamine-like compounds. As detailed in the attached copy of the Ph.D. thesis of M. D. Fairchild, there appeared to be marked variations in the order of the effects of the substituted amphetamine derivatives on transcallosal conduction and in the order of their hallucinogenic activity in man. However, their relative capacity for inhibiting ongoing spontaneous electrical activity in the brain of this anesthetized cat preparation apparently did have some correlation to their hallucinogenic potency in man.

Because of the possibility that changes produced by the test compounds in the spontaneous brain electrical activity of the cat might relate to their capacity for producing hallucinations in man, an

investigation was undertaken into their effects on the unanesthetized and unrestrained animal with chronically implanted recording electrodes. The compounds were found to produce a characteristic hypersynchronous bursting pattern in this preparation and the study of such activity made up the main substance of our investigation of Electroencephalographic Test Methods under our contract.

The development of an 8-channel frequency analyzer was undertaken in an attempt to quantify the amount of hypersynchronous bursting activity produced by various doses of the test compounds. The details of the completed apparatus are given in the Ph.D. thesis attached. We were successful in obtaining semi-quantitative results which indicated that MDA and TMA were the most potent compounds in the series. DMA was found to be the least potent and MMDA occupied a position midway between the least and most active compounds. The activities of mescaline and DMA were of a similar order while amphetamine did not produce hypersynchronous bursting in the electroencephalogram of the conscious cat. This order of activity is approximately similar to that found in man for producing hallucinations and therefore the method holds some promise as a potential tool for investigating in experimental animals certain compounds which have psychotropic actions in man.

No attempt was made during these investigations to generalize the information beyond the results obtained with the six test compounds. However, it would be of interest to ascertain whether other series of "hallucinogenic" agents are capable of producing hypersynchronous bursting activity in the brain of the conscious cat and whether the quantitative aspects between the members of the series would be ordered

in a fashion similar to that found in man. Another interest would be to test the effects of "tranquillizing" agents on the hypersynchronous bursting activity. Such experiments would be necessary before the method outlined in this work could be thought to have a more general usefulness as a psychopharmacological tool.

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ABSTRACT OF THE DISSERTATION

Some Central Nervous System Effects of Four

Phenyl-Substituted Amphetamine

Derivatives

by

Mahlon David Fairchild

University of California, Los Angeles, 1963

Professor in Residence, Gordon A. Alles, Chairman

Among the numerous natural and synthetic chemicals which ef-
fect the central nervous system there exists a group of compounds
distinguished by their ability to produce hallucinations in man.
Certain similarities between the mental state induced by these drugs
and that existing in some forms of endogenous psychoses has inter-
ested medical investigators.

Because of the subjective nature of their effects the so-call-
ed "hallucinogenic" agents have largely been investigated using man
as the experimental subject. The present work represents an attempt
to employ sub-human species in the study of a series of hallucino-
genic compounds. Empirical correlations were sought between the po-
tency of the test compounds for producing certain behavioral and
neurophysiological changes in mice and cats and their known potency
for eliciting hallucinations in man.

The test compounds consisted of the following series of

phenyl-substituted amphetamine derivatives: DL-3,4,5-trimethoxy-phenisopropylamine hydrochloride (TMA); DL-3,4-methylenedioxy-phen-isopropylamine hydrochloride (MDA); DL-3,4-dimethoxy-phenisopropyla-mine hydrochloride (DMA) and DL-3,4-methylenedioxy-5-methoxy-phen-isopropylamine hydrochloride (MMDA). They were compared to two related standard compounds: 3,4,5-trimethoxy-phenethylamine sulfate hydrate (mescaline) and DL-phenisopropylamine sulfate (amphetamine).

Among the amphetamine derivatives TMA and MDA are the most potent hallucinogenic agents in man; DMA is the least potent. No direct information is available concerning the hallucinatory effects of MMDA although estimation of activity from sub-hallucinatory doses would place it between the most and least active compounds. With regard to the related standard compounds, mescaline has a potency comparable to DMA while amphetamine is not regarded as a hallucino-genic agent.

The potency of the test compounds for inhibiting a trans-callosal evoked potential in the brain of anesthetized cats had no correlation to their hallucinogenic activity in man.

The ability of mescaline and the amphetamine derivatives to cause a rapid extinction of a conditioned avoidance response in mice did correlate with their hallucinogenic potency. However, ampheta-mine, which is not a hallucinogenic agent, produced the same effect.

Mescaline and the amphetamine derivatives, but not amphetamine itself, elicited a characteristic hypersynchronous bursting activity in the electrographic recordings from the cortex and various deep

structures within the brain of the conscious cat. A quantification of the frequency analysis from the dorsal hippocampus revealed that the potency of the test compounds for producing this response correlated well with their hallucinogenic activity in man.

Suggestions are made for future research in these areas.

INTRODUCTION

Among the natural and synthetic chemical compounds ingested
by man in the course of history there exists an array of substances
which are distinguished by their ability to alter his mental state.
Such centrally active compounds include a group of drugs, the hallu-
cinogens, which are able to produce changes in mental functions that
resemble the endogenous psychotic states and have captured the inter-
est of growing numbers of medical investigators. The reason for this
interest is fairly obvious. Psychosis, in its many forms, is one of
man's most grievous afflictions and yet its etiology is essentially
unknown. When chemicals of known structure can reproduce some of the
symptoms of these diseases, investigations into their properties
seems a fruitful area of study.

The recent clinical introduction of another class of centrally
active compounds, now commonly termed tranquilizers, has strongly
reinforced the possibility of a chemotherapeutic approach to the cure
of mental illness. These drugs have revolutionized the treatment of
psychosis because they are able to markedly alter processes respon-
sible for psychotic behavior.

The concept of a drug induced "model psychosis" and its pos-
sible role in elucidating mechanism of action and therapeutic proce-
dures in mental diseases has been the subject of much research effort
and controversy in recent years. Some investigators strongly main-
tain that there is little similarity between the drug induced and
endogenous psychotic states (cf. Hollister, 1962) while others be-

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lieve that the two are closely related (von Mering, Moromito, Hyde and Rinkel, 1957; Greinor, Burch and Edolbert, 1958). A more moderate approach (Denber, 1962) would be that sufficient common symptoms exist to warrant continuing efforts at study along these lines.

While hallucinogens and tranquilizers produce a number of pharmacological responses their subjective effects in man have been of major interest. However, use of experimental animals would greatly facilitate pharmacological and clinical research if some reasonable degree of correlation could be found between measurable parameters in such animal work and the subjective effects of the drugs as experienced by man.

The present studies are concerned with some neurophysiological and behavioral effects caused by a series of centrally active compounds in two sub-primate mammalian species. Certain of these compounds are known to have different degrees of potency for producing hallucinatory phenomena in man. The major objective of the study was to seek correlation between the relative potency exhibited by these compounds for producing objectively measurable effects in animals and their reported potency for producing hallucinations in man.

The compounds, consisting of four phenyl-substituted amphetamine derivatives will be described in detail in Chapter I. This particular series of derivatives was chosen because it represents a group of compounds which differ only slightly in their chemical structure and properties but which exhibit qualitative and quantitative differences in their effects on the central nervous system. It

was felt that their relatively small molecular structural changes might provide an opportunity to gain information regarding structure-activity relationships.

The techniques employed to gain quantitative information on the effects of these compounds in experimental animals and the relationships between the results obtained and hallucinogenic phenomena in man will be discussed in Chapters II, III and IV.

Chapter V will be devoted to a discussion of general considerations and conclusions arising from the experimental work and proposals for future investigations in this field.

THE TEST COMPOUNDS

A. __INTRODUCTION__

One of the amphetamine derivatives to be employed in these investigations can be regarded as a combination of the trimethoxy substituted phenyl nucleus of mescaline with the isopropyl side chain of amphetamine. In terms of the structural formula the relationships can be diagrammed as follows:

Trimethoxyamphetamine
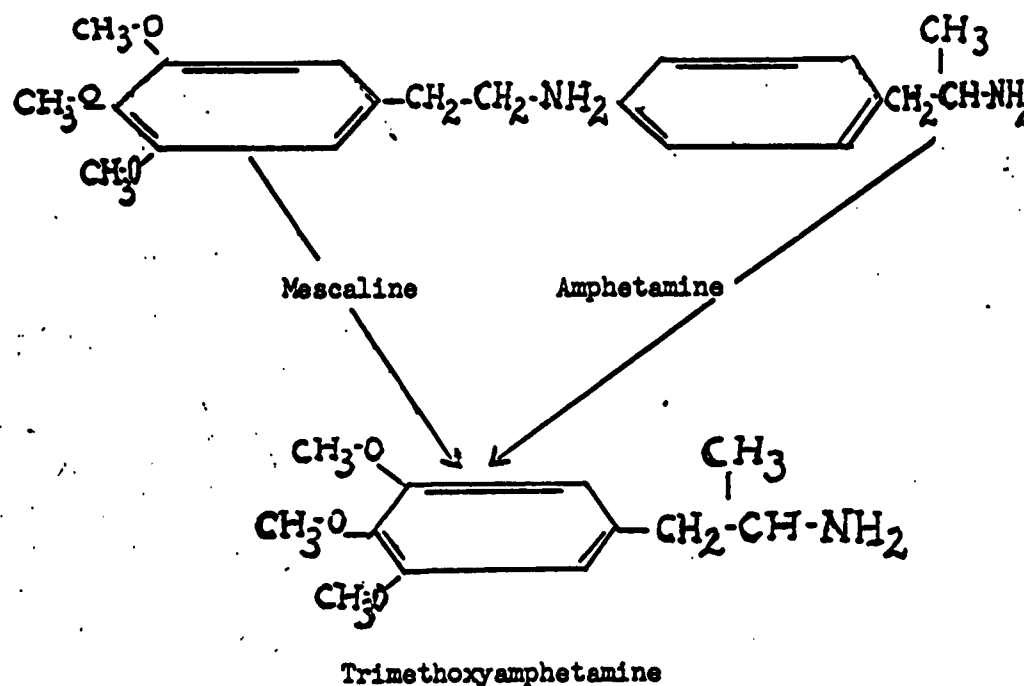
The other three amphetamine derivatives are similar to trimethoxyamphetamine but have different substituents on the phenyl nucleus. All of the amphetamine derivatives contain the isopropyl side chain and for this reason can exist as optical isomers but no attempt was made in this study to separate the isomeric forms and all

compounds were employed in the form of their racemic mixtures (50% dextro and 50% levo).

Because of the relatively long chemical names of these compounds and the numerous references which must be made to them a system of abbreviations was adopted which will be utilized throughout the remainder of this thesis. These abbreviations along with the correct chemical designation are listed in table 1.

While the four amphetamine derivatives were used as the hydrochloride salts, mescaline was employed as its sulfate hydrate and amphetamine as its sulfate salt.

The expression of doses in terms of millimoles per kilogram (mM/kg) is of importance for comparisons made between substances having different molecular weights. It will be recalled that:

millimoles (mM) x equivalent molecular weight = milligrams (mg) .

B. SOME PHYSICAL AND CHEMICAL PROPERTIES

The standard compounds, mescaline and amphetamine, and the four amphetamine derivatives were synthesized and prepared in the form of suitable salts in the Laboratories of Dr. Gordon A. Alles.

1. The Standard Compounds

a) Mescaline in the form of its sulfate hydrate

$$CH_3 \cdot O \underset{CH_3 \cdot O}{\overset{CH_3 \cdot O}{\bigcirc}} -CH_2 \cdot CH_2 \cdot NH_2$$
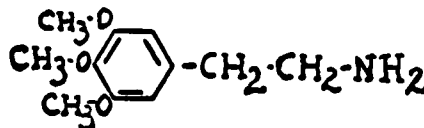
$(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O$ Equivalent Weight 278

TABLE 1

ABBREVIATIONS AND CHEMICAL NOMENCLATURE OF THE TEST COMPOUNDS

The Phenyl-Substituted Amphetamine Derivatives

Chemical Name	Abbreviation
DL-3,4,5-trimethoxy-amphetamine DL-3,4,5-trimethoxy-phenisopropylamine DL-a-methyl-3,4,5-trimethoxy-phenethylamine DL-a-methyl-mescaline	TMA
DL-3,4-methylenedioxy-amphetamine DL-3,4-methylenedioxy-phenisopropylamine DL-a-methyl-3,4-methylenedioxy-phenthylamine	MDA
DL-3,4-dimethoxy-amphetamine DL-3,4-dimethoxy-phenisopropylamine DL-a-methyl-3,4-dimethoxy-phenethylamine	DMA
DL-3,4-methylenedioxy-5-methoxy-amphetamine DL-3,4-methylenedioxy-5-methoxy-phenisopropylamine DL-a-methyl-3,4-methylenedioxy-5-methoxy-phenethylamine	MMDA

The Related Standard Compounds

Chemical Name	Generic Name
3,4,5-trimethoxy-phenethylamine	mescaline
DL-phenisopropylamine DL-a-methyl-phenethylamine	amphetamine

The naturally occurring base was isolated from the peyote cactus Lophophora Williamsii and established as its principal centrally active constituent by Heffter (1894). It was later synthesized by Späth (1918).

b) Amphetamine in the form of its sulfate

$$\langle\bigcirc\rangle\text{-CH}_2\text{-CH-NH}_2 \quad \overset{CH_3}{|}$$
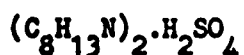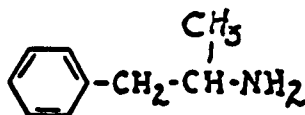
$(C_8H_{13}N)_2\cdot H_2SO_4$ Equivalent Weight 184

Prepared as a base for chemical study by Edeleano (1887) and later in the form of its salts by Alles (1932) who found them to have some notable central and peripheral activities (1933).

2. The Amphetamine Derivatives

a) TMA — Trimethoxy-amphetamine as its hydrochloride

$$\begin{array}{c} CH_3O \\ CH_3O \\ CH_3O \end{array} \langle\bigcirc\rangle\text{-CH}_2\text{-CH-NH}_2 \quad \overset{CH_3}{|}$$

$(C_{12}H_{19}NO_3)\cdot HCl$ m.p. 217-8°   Equivalent Weight 262
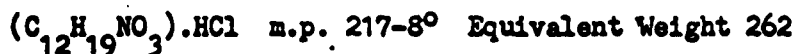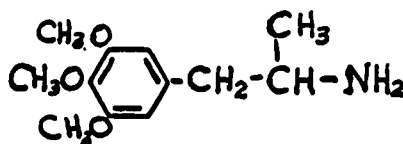
Synthesis of this compound was reported by Hey (1947) and it had been used by Alles and Heegaard (1943) for a study of the specificity of amine oxidase.

b)   MDA — Methylenedioxy-amphetamine as its hydrochloride

$$O \cdot \underset{CH_2O}{\overset{}{\bigcirc}} -CH_2 \cdot \overset{CH_3}{\underset{}{CH}} \cdot NH_2$$
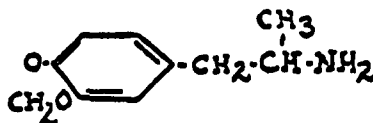
$(C_{10}H_{13}NO_3).HCl$ m.p. 186-7°   Equivalent Weight 216

Synthesis and properties of this compound were reported by Mannich and Jacobsen (1910) and it was later used by Alles and Heegaard (1943) for a study of the specificity of amine oxidase.

c)   DMA — Dimethoxy-amphetamine as its hydrochloride

$$\underset{CH_3O}{\overset{CH_3O}{\bigcirc}} -CH_2 \overset{CH_3}{\underset{}{CH}} -NH_2$$
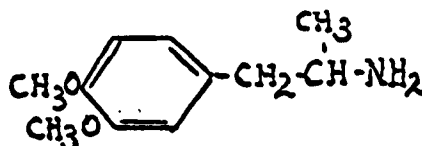
$(C_{11}H_{17}NO_2).HCl$ m.p. 147-8°   Equivalent Weight 232

Synthesis and properties of this compound were reported by Mannich and Jacobsen (1910) and again by Alles (1932). It was later used by Alles and Heegaard (1943) for a study of the specificity of amine oxidase.

d)   MMDA — Methoxy-methylenedioxy-amphetamine as its

hydrochloride

$$\underset{CH_2O}{\overset{CH_3O}{\underset{O}{\bigcirc}}} -CH_2 - \overset{CH_3}{\underset{}{CH}} - NH_2$$
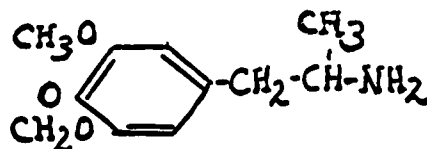
$(C_{11}H_{15}NO_3).HCl$ m.p. 178-9°   Equivalent Weight 246

MMDA is a new compound synthesized by Alles

(1962) and has not previously been reported in the
·literature. It was prepared by lithium aluminum hy-
dride reduction of 5-methoxy-3,4-methylenedioxyphenyl-
nitro-propylene obtained by the condensation of nitro-
ethane with myristic aldehyde. This sequence of syn-
thesis corresponded to that reported by Hoy (1947) for
the synthesis of TMA. The sample of MMDA was of its
hydrochloride, recrystallized from hot ethanol, melt-
ing at 177-178°C. and showing chloride analysis of
14.39% as compared with 14.43% calculated for
$(C_{11}H_{15}NO_3)$.HCL of molecular weight 245.71.

C. HALLUCINATORY EFFECTS IN MAN

1. The Standard Compounds

a) Mescaline

The reaction of man to mescaline administration .
has had extensive documentation. Lewin was one of the
first investigators to become interested in the physi-
ological activity of various fluid extracts of the
cactus plant then known as Anhalonium lewinii (now
Lophophora williamsii). In a paper published in 1888
he described the effects of injecting these extracts
into various experimental animals and concluded that
he was "dealing with an intensely poisonous substance".
Lewin's publications (1887, 1888, 1894) in a sense
introduced "peyote" into modern western medicine.

This cactus has been used for centuries by certain Mexican Indians in conjunction with various religious ceremonies. The drug was in widespread use by the Aztecs when the Spaniards arrived in 1520 and was worshipped among the triad of plants known as "teonanacatl, ololiuqui and peyotl." (De Ropp, 1957). Today the religious use of peyote survives in the Native American Church, a christian sect having branches in Mexico, in the southwestern United States and in eastern Canada. The eating of peyote has been incorporated into the ceremonies of this church and this practice has come under sharp criticism. Five anthropologists (La Barre, McAllester, Slotkin, Stewart and Tax, 1951) investigated the Greater American Church and their findings amount to a vigorous defense of the use of peyote by this group. They denied any abuse of the drug and concluded that:

> . . . It will be seen from this brief description that the Native American Church of the United States is a legitimate religious organization deserving the same rights to religious freedom as other churches; also that peyote is used sacramentally in a manner corresponding to the bread and wine of the white Christian.

Around the turn of the century a number of reports appeared in the medical literature which were concerned with the mental changes produced by ingesting peyote. Prentiss and Morgan (1895) described in

considerable detail the effects reported by 5 individuals following ingestion of "mescal buttons" and concluded that "visions" and muscular depression were the main changes produced by the drug. It was noted that the visions ranged from only flashes of color to the most complex and detailed panoramas; that they occurred most frequently with the eyes closed and usually without any concomitant clouding of consciousness. Mitchell (1896), following the self-administration of an "extract" of peyote, experienced a variety of detailed visual hallucinations which were preceded by less complex lines and patterns of color. Klüver (1928) pointed out that certain "hallucinatory constants" consisting of geometrical forms and patterns seemed to occur with marked regularity during the course of peyote intoxication. These "constants" were then replaced by more variable "panoramic" visions as the drug effect became more pronounced.

The alkaloid primarily responsible for the central effects of peyote is mescaline which was isolated by Heffter as early as 1894. Practically all medical investigations conducted in this field within the last several decades have employed mescaline as the pure compound rather than using the crude plant preparation.

A number of studies are available for estimating the dose of mescaline necessary to produce hallu-

cinatory phenomena in man. In one of the most recent articles on the subject Hollister and Hartman (1962) reported that 5 mg/kg of mescaline (probably the sulfate hydrate), given orally to 20 individuals judged to be psychiatrically "normal", produced visual hallucinations in 9, hallucinations of smell or taste in 3 and auditory hallucination in 2 of the cases. A total of 17 out of the 20 subjects reported alterations in perception of shapes or colors. Hoch (1951) and Hoch, Cattell and Pennes (1952) reported that intravenous doses of 400-600 mg mescaline sulfate (8 to 12 mg/kg or .029 to .043 mM/kg) in 59 mental patients produced visual hallucinations in 44, auditory hallucinations in 18 and hallucinations of smell or taste in 7 individuals. Feigen and Alles (1955) working with smaller doses of mescaline sulfate found that with oral administration of 100, 150 and 200 mg (2 to 4 mg/kg or .007 to .014 mM/kg) only 3 of 7 individuals tested experienced hallucinations at the 200 mg dose level. These investigators also measured the blood pressure, pulse, heart rate, areas of visual color fields and patellar reflex strength. The clearest change produced by mescaline, even at sub-hallucinatory doses, was pupil dilation and an increase in the patellar tendon reflex, which apparently indicated an elevated reflex excitability. Areas of the visual color

fields, especially in the blue spectrum, were in-
creased with higher doses of the drug. ~~Cardiovascular~~
findings were essentially negative. Beringer (1927),
in a study involving some 60 subjects, concluded that
the individual responses to mescaline were so variable
that comparative studies would require administering a
range of doses to each subject in order to derive the
reaction spectrum.

To summarize, hallucinatory phenomena might be
expected to occur in most individuals with doses in
the neighborhood of 5-10 mg/kg (.018-.036 mM/kg) of
the sulfate hydrate salt.

b) Amphetamine

This compound is a potent central nervous sys-
tem stimulant. In most individuals 5 mg of the d-iso-
mer, taken orally, will produce a marked subjective
stimulation which is unaccompanied by other gross
physiological changes. At doses of 40 mg and higher
it will produce increased blood pressure, mydriasis
and other "sympathomimetic" responses but in doses of
20 mg and less these effects are usually of little
consequence. Alles (1939) reported that the d-isomer
of amphetamine was approximately 4 times more potent
than the 1-isomer for producing central stimulation in
man although these two optically active forms and
their racemic mixture were found to have comparable

pressor effects.

Whether or not amphetamine produces hallucina-
tory phenomena when taken in high doses is a complex
question. Monroe and Drell (1947) studied an army
prison population which had relatively unrestricted
access to amphetamine through the use of Benzedrine
Inhalers. In a questionnaire returned by 1082 inmates
25% admitted regular use of amphetamine. The authors
stated that this use gave rise to serious disciplinary,
medical and psychiatric problems but did not discuss
the nature of these problems to any extent. Only 4 of
approximately 270 men admitting amphetamine use were
prompted to seek medical help as a result of taking
the drug. These individuals had developed a psychotic
paranoid state. This relatively low incidence of
severe mental disturbance occurred in a population
which could be considered unstable by its nature and
where the only source of amphetamine was from inhalers
each of which contained 325 mg of the free base.

The question of serious mental disturbances re-
sulting from the use of amphetamine was the subject of
a monograph by Connell (1958). This author, from a
detailed review of the English and French literature,
concluded that either chronic or acute high doses of
amphetamine are capable of producing a paranoid
schizophrenic-like state in certain individuals.

Connell himself studied 42 cases of psychosis in which
amphetamine was involved. The history of these indi-
viduals indicated a marked degree of mental instabil-
ity. A total of 66% had been previously treated for
some type of severe emotional disturbance, 20% were
classified as psychoneurotics and only 14% were con-
sidered to have relatively "normal" backgrounds and
personalities. The symptoms exhibited by these pa-
tients, in agreement with cases reported in the liter-
ature, showed a high degree of paranoid reactions
which in many instances were associated with hallu-
cinatory phenomena. The author points out that 9 of
these patients developed psychosis after a large dose
of amphetamine taken over a short period of time. The
dosage range in this group was from 75 mg of d-amphet-
amine taken as a single dose, to 975 mg of the base
taken over a 3-day period. From these results, Con-
nell concluded that amphetamine can be considered as a
true hallucinogenic agent. A major objection to this
conclusion was recognized by Connell himself and
stated in the opening paragraph of Chapter II of his
book:

> In a study of this kind, based on the
> premise that a condition is rare and
> associated with excessive doses of a
> drug, it is not possible to proceed
> according to scientific principles and
> use random samples or mixed groups.

While amphetamine in high acute doses has been demonstrated to cause hallucinations, this apparently only usually occurs in individuals with relatively unstable mental histories. No information is available concerning what effects might be expected in a "normal" population. The study of Monroe and Drell, which has been previously discussed, seemed to indicate that even in an "unstable" population relatively high doses of amphetamine produced a comparatively low incidence of mental disturbance of a degree of severity which would involve the production of hallucinations.

In summary, the available data seems to indicate that amphetamine, in high doses, is capable of precipitating a paranoid-schizophrenic-like state which can be accompanied by hallucinatory phenomena. This reaction usually occurs in individuals with unstable mental histories and though it has been reported in some "normal" subjects the incidence in this latter group could be expected to be low. This would not seem sufficient evidence to classify amphetamine as a hallucinogenic agent in man.

2. The Amphetamine Derivatives

   a) TMA

Peretz, Smythies and Gibson (1955) reported that doses of 1.6 to 2.0 mg/kg of the hydrochloride

salt produced visual hallucinations in 9 "normal" in-
dividuals. The hallucinations were very similar to
those reported for mescaline; they were highly colored
and involved geometrical patterns, animal forms, land-
scapes, etc. A stroboscope was used to elicit these
phenomena. With lower doses of TMA (0.8 to 1.2 mg/kg)
no hallucinations occurred but at 1 to 2 hours sudden
"giddiness" developed which was followed by increased
talkativeness and locomotor activity. Definite sensi-
tization of deep tendon reflexes were noted. This
observation is similar to that of Feigen and Alles
(1955) for sub-hallucinatory doses of mescaline.
Alles (1959), in further self-experiments with TMA,
reported that no subjective effects occurred with .2
mg/kg but with a dose of .4 mg/kg they became quite
apparent. A slight, but persistent, gastro-intestinal
disturbance was noted which was paradoxically accom-
panied by a feeling of euphoria. Alles felt aware of
a "broader-than-usual" visual field, although no
mydriasis was evident. In previous experiments he
had found 2.0 mg/kg of mescaline sulfate necessary to
elicit subjective effects which would indicate that
TMA is approximately 5 times more potent than the for-
mer compound for producing threshold subjective ef-
fects. Shulgin, Bunnell and Sargent (1961), using
doses of 1.6 to 2.0 mg/kg TMA, reported somewhat

similar effects to those described by Peretz,

Smythies and Gibson (1955), although with higher

doses (2.8 to 3.5 mg/kg) some symptoms were encounter-

ed which had not previously been reported. In addi-

tion to visual hallucinations and associated phenomena

5 subjects receiving this dose level showed hostile or

"megalomanic euphoric" reactions. These behavioral

changes were severe enough to prompt the authors into

stating that they believed some of these individuals.

could easily have been pushed into a "homicidal fury."

The sensorium was clear during these episodes and re-

call was excellent.

TMA, as judged by the various investigations

discussed above, can apparently cause hallucinations

in man at a dose in the neighborhood of 2.0 mg/kg of

the hydrochloride salt (.088 mM/kg). A hypothetical

ED_{100} (dose producing effects in 100% of the cases)

for mescaline in man might be as high as 10 mg/kg.

This would indicate that TMA is approximately 5 times

more potent than mescaline for producing hallucina-

tions. This figure would agree with the estimate

given by Alles for eliciting threshold subjective

effects in himself.

b) MDA

There is only one report in the general literature

concerning the central effects of MDA in man.

Alles (1959), after ingesting a total dose of 126 mg
of the hydrochloride salt (.006 mM/kg), experienced
visual hallucinations consisting of many grey, curling
smoke rings. These were seen in the environment when-
ever "a relaxed approach to subjective observation was
used" but they disappeared when attempts were made to
concentrate on their detail. Interestingly enough, an
increased awareness of color, so common during mes-
caline and TMA intoxication, did not occur. In addi-
tion to visual hallucinations Alles reported an in-
creased ability to differentiate certain sounds (foot-
steps away from general traffic noises in a busy
street) which was apparently not linked to an over-all
increase in auditory acuity. A pronounced increase in
perception of distant objects and certain feelings of
depersonalization were also noted. Physical symptoms
included maximal mydriasis, increased blood pressure,
muscular tremor and tension and a degree of ataxia.

On the basis of the above report it seems rea-
sonable to assume that MDA is a more potent hallucino-
genic agent than mescaline. Alles (1959) reported
that, in self experiments, 200 mg of mescaline sulfate
were required to produce threshold subjective effects
while only 80 mg of MDA were necessary for similar
activity.

c) DMA

The only account of hallucinogenic phenomena
resulting from DMA, of which the author is aware, is
contained in an Army Chemical Center report (1962).
Under auspices of this group this compound was admin-
istered to several psychiatric patients in the New
York State Psychiatric Institute. One patient re-
ceived .004 mM/kg (.87 mg/kg) of the hydrochloride
salt intravenously and exhibited only a slight in-
crease in psychiatric symptoms; a comparable dose in a
second individual also elicited only insignificant
changes. When one of these two patients was reinjec-
ted at a later date with approximately .04 mM/kg
(10 mg/kg) of DMA a definite "mescaline-like" state
was induced. The symptoms included colored hallucina-
tions of geometrical figures and occasional structured
forms. The other individual experienced visual dis-
tortions, notable after-imagery, feelings of unreality
and paranoid ideas. Marked mydriasis and gross body
tremors also occurred but apparently no hallucinations
were experienced. Alles (1962) has performed self-
experiments with DMA and reports that oral doses from
10 to 120 mg (.0004 to .0050 mM/kg) were without
peripheral or subjective effects, except for a slight
gastro-intestinal discomfort at the higher doses.
When 160 mg (.007 mM/kg) was ingested a 20 mm. of
mercury increase in blood pressure occurred within 45

minutes, which was accompanied by a slight mydriasis, lacrimation, and gastro-intestinal uneasiness. Alles judged this effect to be approximately equal to that of 60-80 mg MDA and concluded that DMA had from 30 to 50% of the potency of the former compound. Fellows and Ullyot (1951) studying the analgetic properties of a large series of compounds mentioned that DMA produced marked analgesia in cats at doses of 20 mg/kg but that clinical testing had indicated the compound was not useful in man. Unfortunately, no dosage details were given for the clinical testing.

On the basis of the rather limited data available it would seem that DMA is less potent than either TMA or MDA for producing hallucinations in man. Its effective dose might be similar to that for mescaline.

d) MMDA

The only information available regarding the central effects of MMDA in man are some self-experiments conducted by Alles (1962) who found that doses of 10 to 40 mg (.0004 to .0016 mM/kg) produced no significant effects. A dose of 80 mg (.0032 mM/kg) MMDA resulted in a slight increase in blood pressure and heart rate plus definite mydriasis although no subjective changes could be discerned. Alles concluded that MMDA had one-half or less the activity of TMA for threshold effects.

3. Summary of Hallucinatory Effects in Man

    a) The Standard Compounds

            Since its introduction into the modern world
    near the turn of the century numerous reports have
    described the hallucinations produced by mescaline.
    The doses used in these studies varied over a rela-
    tively wide range but from the available data it
    seems reasonable to assume that 10 mg/kg of mescaline
    (.036 mM/kg of the sulfate hydrate) taken orally would
    elicit visual hallucinations in most individuals.
    This relatively high dose would also probably produce
    a number of peripheral symptoms including nausea and
    vomiting.

            Amphetamine, while having potent central stimu-
    latory actions, probably should not be classified as a
    hallucinatory agent in man. High doses of this com-
    pound evidently can produce toxic psychotic states
    accompanied by hallucinations in certain individuals
    but it appears that the incidence of such a reaction
    to an acute high dose in a "normal" population is
    relatively low.

    b) The Amphetamine Derivatives

            TMA is the only compound among the four deriva-
    tives which has been reported to produce hallucina-
    tions in more than one "normal" subject. The effec-
    tive dose was in the region of 2.0 mg/kg (.0076 mM/kg

of the hydrochloride salt) Alles reported experiencing hallucinations after ingesting a total dose of MDA equivalent to 1.4 mg/kg (.006mM/kg of the hydrochloride salt). Apparently TMA and MDA have comparable potencies for producing hallucinations in man which is approximately four to five times greater than that reported for mescaline.

DMA at 10 mg/kg produced a typical "mescaline-like" response in one psychotic patient and on the basis of threshold effects Alles judged this compound to be at least 2 to 3 times less active than MDA. DMA may have a potency in man more comparable to mescaline than to MDA or TMA.

No information is available concerning the hallucinatory properties of MMDA. On the basis of threshold effects Alles estimated that the potency of this compound might fall somewhere between DMA and the more active MDA and TMA.

CHAPTER II

THE EFFECTS OF THE AMPHETAMINE DERIVATIVES

ON AN EVOKED POTENTIAL

IN THE BRAIN OF CATS

A. INTRODUCTION

This work was conducted in the Veterans Administration Research Laboratories in Neuropsychiatry in Pittsburgh, Pennsylvania through the kind invitation of Dr. Amedeo S. Marrazzi, Director. The experimental procedures were under the direct supervision of Dr. Ross E. Hart to whom the author owes a special thanks for his patient and thorough instruction.

Drs. Marrazzi and Hart have intensively studied the effects of various pharmacological agents on synaptic transmission within the central nervous system using the "transcallosal preparation" in the cat. Briefly this procedure consists of delivering an electrical pulse to a cortical locus and recording the evoked potential transmitted via the corpus callosum to the homolateral point on the opposite hemisphere. Drug effects are assayed by observing the changes elicited in this evoked response following close intracarotid injections of the various compounds. Marrazzi (1953) discussed the reciprocal nature of epinephrine and acetylcholine actions on this system and pointed out that similar results had been attained with isolated peripheral ganglia. Acetylcholine in doses of 1.0 microgram/kg caused an increase in the height of the transcallosal evoked potential, while epinephrine at 10 micrograms/kg produced a definite de-

crease in the response.

Various psychoactive agents were investigated for their effect
on central synaptic transmission. In 1952 Hart and Marrazzi reported
that 5 mg/kg of mescaline (.018 mM/kg of the sulfate hydrate) injec-
ed into the ipsilateral carotid artery produced marked inhibition of
central synaptic transmission. A dose of 15 mg/kg had similar ef-
fects when injected intravenously. In a subsequent publication
Marrazzi and Hart (1955a) reported that 8 micrograms/kg of LSD-25 and
2 micrograms/kg of serotonin also inhibited the central synapse.
They proposed that a "balanced reciprocal relationship" may normally
exist between cholinergic excitatory and adrenergic inhibitory mech-
anisms and suggested that serotonin, which is endogenous and capable
of producing synaptic inhibition in minute amounts, might play some
role in naturally occurring psychoses by acting to unbalance this
system. These authors (Marrazzi and Hart, 1955b) pointed out that
certain structural similarities exist between the molecule of LSD and
serotonin and speculated on the possibility that both could influence
similar central mechanisms. Further work along these lines (Marrazzi
and Hart, 1956) demonstrated that the tranquillizing agents chlorpro-
mazine, reserpine and azacyclonal were capable of reducing the de-
pressive effects of mescaline in the transcallosal preparation.
Bufotenine (dimethyl serotonin) and adrenochrome, both of which have
been reported to produce hallucinations in man, were also demonstrat-
ed to inhibit central synaptic transmission in the cat. In addition
the relative dose-response relationships among these hallucinatory
agents and their antagonism by the tranquillizers was similar to that

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observed in man.

Because various hallucinogenic agents had been reported to produce synaptic inhibition in the transcallosal preparation in doses which were relative to their potency in man the idea was conceived to compare mescaline and the four amphetamine derivatives in the same preparation. It was hoped that similar correlations could be made with this series of test compounds.

B. PROCEDURE

The experimental animals were cats of either sex weighing between 2 and 4 kilograms. They were initially anesthetized with 30 mg/kg pentobarbital sodium injected intraperitoneally. Additional doses of the anesthetic were given as needed during the course of the experiment by injections into an exposed femoral vein.

A femoral artery was isolated, cannulated and connected to a mercury manometer which recorded blood pressure changes on a kymograph.

A medial longitudinal incision was made in the skin of the neck and the trachea cannulated in the usual manner. Two lateral, dorsally directed incisions were extended from either ends of the medial cut and the skin was layed back as a flap. The external jugular vein was isolated from the underlying tissue and the strap muscles were tied firmly near their origins and insertions. These muscles were removed following incision between the ligations. The carotid artery, thus exposed, was dissected free from the accompanying nerve and fascia. Injections were made directly into this ex-

posed vessel with a sharp 27 gauge needle.

After fixing the animals head in a holder the skull was ex-
posed and an extensive craniotomy was performed. The entire parietal
bone was removed with the exception of a thin strip on either side of
the saggital suture covering the medial sinus. The craniotomy ex-
tended forward into the frontal bones and sinuses and ventrally to
approximately the level of the occipital bones. The dura was removed
and the exposed cortex flooded with warm mineral oil.

An electrode holder was positioned directly over the exposed
cortex by firmly clamping it to the lateral margins of the occipital
bone. The stimulating electrode was positioned along the medial gy-
rus near the midline on one side of the brain and a recording elec-
trode was placed on a homolateral point on the opposite cortex. An
"indifferent" electrode was placed on the hemisphere containing the
recording electrode at a point well removed from it. A square wave
of 10 volts potential and 5 milliseconds duration was applied at the
rate of two per second to the stimulating electrode through a stimu-
lus isolation unit. The potential difference this stimulus evoked
between the recording and indifferent electrodes was amplified and
displayed on the face of a cathode-ray oscilloscope. It was also re-
corded on an inkwriting oscillograph whose primary function was moni-
toring the ongoing spontaneous electrical activity of the brain.

If the evoked potential produced by the 10 volt stimulus was
100 microvolts or greater and if the spontaneous electrical activity
was sufficiently depressed by the barbiturate so as not to unduly in-
fluence the stability of the evoked response the preparation was

deemed satisfactory. It was frequently necessary to "explore" with the recording electrode in an attempt to find a more pronounced evoked potential. Additional barbiturate was often given to reduce the amount of spontaneous electrical activity and thus minimize distortion of the evoked potential. Having obtained a satisfactory response the stimulating voltage was reduced to near threshold level and the frequency was reduced to one per second. A number of "control" traces were photographed from the face of the oscilloscope using a camera with a film speed of 10 mm. per second.

The drugs were then injected directly into the exposed common carotid artery on the same side as the recording electrode and a photographic record of their effect was obtained. The image of each trace was projected from the 35 mm. film onto a grid screen. The height of the negative deflection of the evoked potential above an arbitrarily chosen point was measured. These values were sequentially plotted with the ordinate representing potential height and the pre- and post-injection plots were then compared. If a significant reduction and subsequent recovery of the height of the evoked potential occurred that particular dose of the drug was judged inhibitory to synaptic transmission in the transcallosal preparation. An increase in the height of the potential would have signaled a facilitation of synaptic transmission but this effect was rarely observed during this series of experiments. Mescaline was used as the standard compound. The potency of the amphetamine derivatives for inhibiting central synaptic transmission was expressed as a percentage of a comparable dose of the standard.

The initial dose of mescaline was usually .0025 mM/kg. If this failed to produce a clear inhibition of the evoked potential, twice this amount was injected. This doubling dose procedure continued until at least two doses of mescaline were recorded, one of which produced a definite inhibition.

Following mescaline the particular amphetamine derivative being studied was injected at a dose equivalent to the effective mescaline dose. Once again, if no inhibition occurred a dose twice as large was administered. After obtaining a clear response with the test compound the animal was recalibrated to mescaline.

In most experiments the preparation was given a series of serotonin injections of .00062 to .0018 mM/kg prior to investigating the effects of mescaline and the amphetamine derivatives. This procedure reflected an interest of Dr. Marrazzi's and had no direct relationship to the experiments being discussed here. While the serotonin injections conceivably could have influenced the results of subsequent tests it appeared that if the blood pressure levels could be maintained following the marked falls which serotonin produced, these preparations would give results which were essentially similar to animals not receiving this compound.

C. RESULTS

1. Introduction

The results obtained using the transcallosal preparation in the cat to assay mescaline and the four phenyl-substituted amphetamine derivatives will be summarized

from the following standpoints:

1. Inhibition of the transcallosal evoked potential.

2. Cross-tachyphylaxis or "blocking" between the compounds.

3. Effects on the brain's spontaneous electrical activity.

No attempt will be made to present raw data. The actual plots of the evoked potentials were done on graph paper approximately two feet square and the blood pressure and EEG records ran continuously during the course of the experiment. To reduce this large amount of data to a form in which it could be concisely presented would require labor not commensurate with the negative character of the findings.

The sensitivity of the individual preparations was judged by the dose of mescaline which would produce a significant inhibition of the evoked potential; the necessary amounts ranged between .0025 and .0200 mM/kg with most animals responding to .005 mM/kg. The compounds to be compared to mescaline were then injected in increasing doses in an attempt to match the effects of this standard.

The activity of these compounds on blood pressure will not be discussed in detail but, with the exception of MDA all are reasonably potent depressor agents; MDA usually produced a marked rise in blood pressure.

2. <u>Comparative Effects of the Amphetamine Derivatives</u>

a) DMA

(1) Effects on the Evoked Potential

In two experiments DMA proved to be approximately equal to or slightly less effective than mescaline for producing central synaptic inhibition. In a third experiment DMA produced a 100% inhibition which did not spontaneously recover while an equimolar dose of mescaline injected prior to it produced a reversible 65% inhibition.

(2) Blocking Effects

There was a suggestion that DMA may attenuate the effects of subsequent mescaline injections. The results, however, were not clear cut.

(3) EEG Effects

DMA has a definite, but not marked, tendency to quiet the EEG.

b) TMA

(1) Effects on the Evoked Potential

The results of four experiments indicated that TMA has approximately one-half the potency of mescaline for producing transcallosal synaptic inhibition.

(2) Blocking Effects

TMA attenuated, and in one instance seemed to reverse, the effects of subsequent mescaline

injections.

    (3) **EEG Effects**

        TMA has a marked potency in reducing EEG activity.

c) MDA

    (1) Effects on the Evoked Potential

        In one experiment MDA appeared to be approximately equal to mescaline in producing central synaptic inhibition. In a second experiment it exhibited less than 50% of the potency of mescaline.

    (2) Blocking Effects

        Results were not clear but there was a suggestion that MDA attenuated the effects of subsequent mescaline injections.

    (3) EEG Effects

        Both experiments indicated that MDA has a marked potency for reducing EEG activity.

d) MMDA

    (1) Effects on the Evoked Potential

        Four experiments indicated that MMDA has less than one-half the potency of mescaline for producing central synaptic inhibition.

    (2) Blocking Effects

        MMDA attenuates the effects of subsequent mescaline injections.

(3) EEG Effects

MMDA has a definite, but not marked, tendency to quiet the EEG.

D. CONCLUSIONS

1. Inhibition of the Transcallosal Evoked Potential

The primary reference standard, mescaline, seemed to be more potent than any of the amphetamine derivatives for producing a decrease in the height of the negative deflection of the electrically evoked transcallosal potential in the cortex of the cat. DMA was found to have an activity nearly equal to that of mescaline and MDA, TMA and MMDA proved to have approximately one-half the potency of mescaline for inhibiting the evoked potential.

2. Cross Tachyphylaxis or "Blocking"

All four amphetamine derivatives probably produced a cross-tachyphylaxis or blocking effect to subsequent mescaline injections. The evidence for this was reasonably clear in the case of TMA and MMDA while the data on DMA and MDA only indicated the possibility of such a phenomena. Actual reversal of the subsequent effect of mescaline was seen on at least one occasion. Indications of reversals were seen in the plots of three or four other experiments but since this effect was unexpected it often was not completely photographed.

3. Spontaneous Brain Electrical Activity

The ability of the amphetamine derivatives to re-
duce the amplitude of spontaneous electrical activity in
the cortex was a definite and reproducible phenomenon;
both TMA and MDA were especially potent in this regard
with MMDA and DMA being clearly less active. Mescaline
also reduced spontaneous activity but in no experiment was
it more active than the amphetamine derivative being com-
pared to it.

E. DISCUSSION

The surgical procedures necessary for the transcallosal prep-
aration were quite extensive and were carried out under deep barbi-
turate anesthesia; the amount of sodium pentobarbital required varied
to some degree from animal to animal. Once the preparation was com-
pleted and recording begun it was often necessary to inject addition-
al barbiturate in order to reduce the amount of spontaneous electri-
cal activity to a point where the evoked potential could easily be
seen. Again this amount varied from preparation to preparation. In
addition to these considerations the cat was continually metabolizing
the barbiturate during the course of the experiment and thus the
level of this drug within the brain varied with time.

Detailed studies on the effects of barbiturates in the CNS
have largely emphasized the depression produced by these compounds on
the reticular activating system of Moruzzi and Magoun (1949). Brad-
ley and Key (1958), Schallek and Kuehn (1959), and Arduini and
Arduini (1954), among others, have reported this fact. Rinaldi and

Himwitch (1955b) finding that even small doses of barbiturates alter-
ed the normal alerting response in the cortex concluded that, "a
special effect predominately on the cortex may account for part of
the hypnotic action of barbiturates."

An additional observation made during the course of these ex-
periments also indicated that barbiturate levels may be an important
consideration for synaptic transmission within the cortex. In many
preparations the height of the evoked potential was seen to change
significantly following barbiturate administration. Some optimum
level of hypnosis seemed to exist at which the potential was at a
maximum.

It can be argued that the standard compound and the drug being
compared to it would be equally effected by shifting barbiturate
levels and thus a comparative evaluation would be valid. The propo-
sition also could be advanced that the effects of mescaline and the
four amphetamine derivatives on synaptic transmission might conceiv-
ably vary independently at different levels of barbiturate anesthesia.

Progressive decline in blood pressure engendered by repeated
injections of mescaline and "cross tachyphylaxis" between the com-
pounds made it impractical to test more than two or three of these
drugs in a single preparation. Since it was not possible to get
valid comparisons of all compounds within any one animal the role of
variable levels of barbiturate background could not be ascertained.
Although it appeared that this factor did not affect the action of
these drugs in a qualitative sense it is conceivable that it may have
contributed to some of the quantitative variations which were en-

countered.

Several investigators have indicated that mescaline is capable of facilitating rather than inhibiting evoked potentials recorded from the cortex under various conditions. Rovetta (1955) working with cats anesthetized with a chloralose-urethane mixture reported that topical application of mescaline in concentrated solutions (33%) to the primary optic cortex resulted in increases in the amplitude of the cortical response to photic stimulation up to 30 times that for control responses. This author also found similar increases in somato-sensory cortex following sciatic nerve stimulation and in evoked potentials from the auditory cortex following click stimuli. Intravenous injections of 12.5 mg/kg mescaline had an opposite effect on the evoked potential in the optic cortex and produced slight decreases in amplitude. LSD, in the hands of this investigator, had essentially no effect on evoked potentials, either topically applied or intravenously injected. Smythies, Koella and Levy (1960) found that in the unanesthetized rabbit intravenous doses of mescaline from 5 to 40 mg/kg produced facilitation of evoked responses recorded from the optic cortex following photic stimulation. They concluded that large doses of the drug "caused an initial inhibition of the responses and an increase in latency followed by a facilitation and decrease in latency."

Purpura (1956a and b), studying the effects of LSD on various evoked potentials in the brains of cats paralyzed with succinylcholine, found that doses of 20 micrograms/kg given intravenously facilitated primary visual and auditory responses in the cortex. On the

other hand, inhibition of thalamocortical and reticulocortical evoked
potentials was reported following 5 micrograms/kg LSD. Purpura theo-
rized a dual action for LSD on central synapses. Facilitation of
specific afferent pathways occurred at doses producing inhibition of
diencephalic-cortical projections. Marrazzi and Hart (1955a) found
an inhibitory action on transcallosal evoked potentials with similar
doses of LSD injected into the carotid artery.

While the relative evaluation of various hallucinatory and
tranquillizing compounds, established by measuring their effects on
the evoked potential of the transcallosal preparation in the cat,
were reported by Marrazzi and Hart to have similarities to their
activity in man no such relationships were found to exist for this
series of amphetamine derivatives. Mescaline proved to be the most
effective compound for inhibition of the transcallosal evoked poten-
tial and was approximately twice as potent as either TMA or MDA. In
man these latter two compounds are apparently two to three times more
potent than mescaline for producing hallucinations.

The reasons why we failed to obtain positive correlations be-
tween the ability of the amphetamine derivatives to inhibit the
transcallosal evoked potential in the cat and their potency for pro-
ducing hallucinatory phenomena in man is not known, although various
reports in the literature suggest that the activity of mescaline, and
presumably mescaline-like compounds, on evoked potentials in the cen-
tral nervous system may be a relatively complex phenomenon involving
both inhibitory and facilitory mechanisms.

While the relative order of potency for the effect of these

compounds on the evoked potential in the cat may not bear any rela-
tionship to their hallucinatory activity in man it should be pointed
out that their ability to inhibit the ongoing, spontaneous, brain
electrical activity in this species did exhibit such a relationship;
TMA and MDA were the most effective compounds in this regard and they
are also the most potent hallucinogenic agents of this series in man.

F. SUMMARY

The effects of mescaline and the four amphetamine derivatives
on the amplitude of a transcallosal evoked potential were measured in
the brains of anesthetized cats. Mescaline was the most potent com-
pound in the series for inhibiting this response. DMA was only
slightly less effective while TMA, MDA and MMDA had approximately
one-half the potency of mescaline.

The compounds also produced a definite decrease in the ampli-
tude of spontaneous brain electrical activity in these preparations.
TMA and MDA were the most potent compounds in this regard with mes-
caline and DMA being clearly less active. MMDA apparently fell some-
where between the two extremes.

Amphetamine was not tested.

Their relative order of potency for inhibiting the trans-
callosal evoked potential did not correspond to the potency of these
compounds for producing hallucinations in man although their ability
to attenuate ongoing, spontaneous electrical activity did show a
degree of correlation.

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CHAPTER III

THE EFFECTS OF THE AMPHETAMINE DERIVATIVES ON MICE

CONDITIONED TO AN AVOIDANCE-ESCAPE SITUATION

A. INTRODUCTION

Sidman (1959), in the opening statement of the first article
to appear in the new journal "Psychopharmacologia," makes the follow-
ing statement:

> Pharmacological agents that exercise effects on
> behavior have been known since antiquity. But
> intensive and systematic efforts to investigate
> the relations between drugs and behavior have
> only recently begun. A major factor contributing
> to the slowness of development of a science of
> Behavioral Pharmacology was the late recognition
> that behavior is a phenomenon amenable to study
> by the methods of Natural Science. The overt
> behavioral effects of drugs were disguised, in
> conformity with general psychological practice,
> by such terms as "alertness," "stimulation,"
> "euphoria," "sedation" and more recently "tran-
> quilizing." These and other supposedly descrip-
> tive terms provided a catchall classificatory
> scheme in which to deposit a bewildering variety
> of behavioral observations. They serve to impose
> a beguiling but false veneer of simplicity upon
> the behavioral changes associated with the admin-
> istration of chemical agents. Behavioral disorders
> are difficult enough to describe at the human level,
> and in animals, with which we share neither a com-
> mon verbal intercourse nor a common behavioral
> topography, a description in terms of traditional
> classifications is virtually impossible. It is
> apparent that the less precise our behavioral
> specification, the less precise will be our know-
> ledge of the relations between drugs and behavior.

These observations by Sidman are particularly applicable to
any study which contemplates employing drug induced behavioral
changes in experimental animals to investigate compounds which are of
interest primarily because they are capable of producing hallucina-

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tory phenomena in man. The "lack of common verbal intercourse" and "common behavioral topography" renders the design of such an experiment very difficult. No a priori assumptions can be made concerning which, if any, of the possible drug induced changes in animal behavior might meaningfully relate to the subjective human experience of hallucinations. However, certain correlations might exist. For example, in a given series of compounds it might be established that drug A was more potent than drug B which was in turn more effective than drug C for producing hallucinations in man. If this same A-B-C potency relationship held for eliciting a specific behavioral change in animals a useful empirical correlation might be described between the two phenomena. The degree of significance for this correlation would depend on the number of compounds compared, the accuracy which their potency was measured and how closely the ranking of the compounds agreed in the two systems.

The experiments which will be described in this chapter measure the effects of two reference compounds, mescaline and amphetamine, and our series of amphetamine derivatives on a conditioned-avoidance response in mice. An attempt will be made to relate quantitatively the influence of these compounds on this specific behavioral situation to what is known concerning their hallucinatory activity in man.

B. TOXICITY OF THE TEST COMPOUNDS IN MICE

In man the central effects of the test compounds occur at doses which do not normally produce major toxic symptoms. In mice

information concerning the lethality of the drugs would be helpful in estimating the possible extent to which general toxic reactions (as opposed to more specific central effects) might contribute to observed changes in the conditioned response. The LD_{50} (dose lethal to 50%) was determined in the following manner:

A total of 32 to 40 mice of the Swiss, albino strain were used in dosage groups of 8 animals each for determining the LD_{50} and 19/20 confidence limits for each drug. The mice were injected intraperitoneally with graded doses of the test compounds; less than 0.5 ml of the aqueous solution was used in each case. Following injection the animals were housed individually in standard, small animal cages in a room whose temperature was maintained at 72° F. Animals dying within 24 hours were recorded and the results analyzed according to the method of Litchfield and Wilcoxon (1949). The resulting data are summarized in table 2.

C. APPARATUS

The apparatus used to condition the mice is illustrated in figure 1. It consisted of a rectangular, white box 16 inches long, 4 inches wide and 12 inches high, which was divided into equal compartments by a partition across the center of its long axis. A plastic door, 1 1/2 inches square, was suspended from the top edge of a slightly larger opening at the bottom of the center partition. Microswitches mounted on either side of this door were activated when it was pushed approximately 45° from center. Circuit closure by the microswitches in turn activated a writing arm which recorded each

TABLE 2

Intraperitoneal Acute Toxicity in Mice

LD_{50} (19/20 Confidence Limits)

Compounds	mM/kg	mg/kg
Amphetamine*	0.06 (0.05-0.09)	11 (9-17)
Mescaline	0.83 (0.77-0.89)	231 (214-248)
MDA	0.16 (0.11-0.22)	35 (24-47)
TMA	0.38 (0.30-0.48)	99 (78-126)
MMDA	0.65 (0.55-0.77)	160 (135-189)
DMA	0.86 (0.76-0.97)	199 (176-225)

*Housed in groups of 4 rather than singly.

Kymograph
&
Event Marker

Shuttle Box

Grid
Scrambler
&
Shock Unit

Timing &
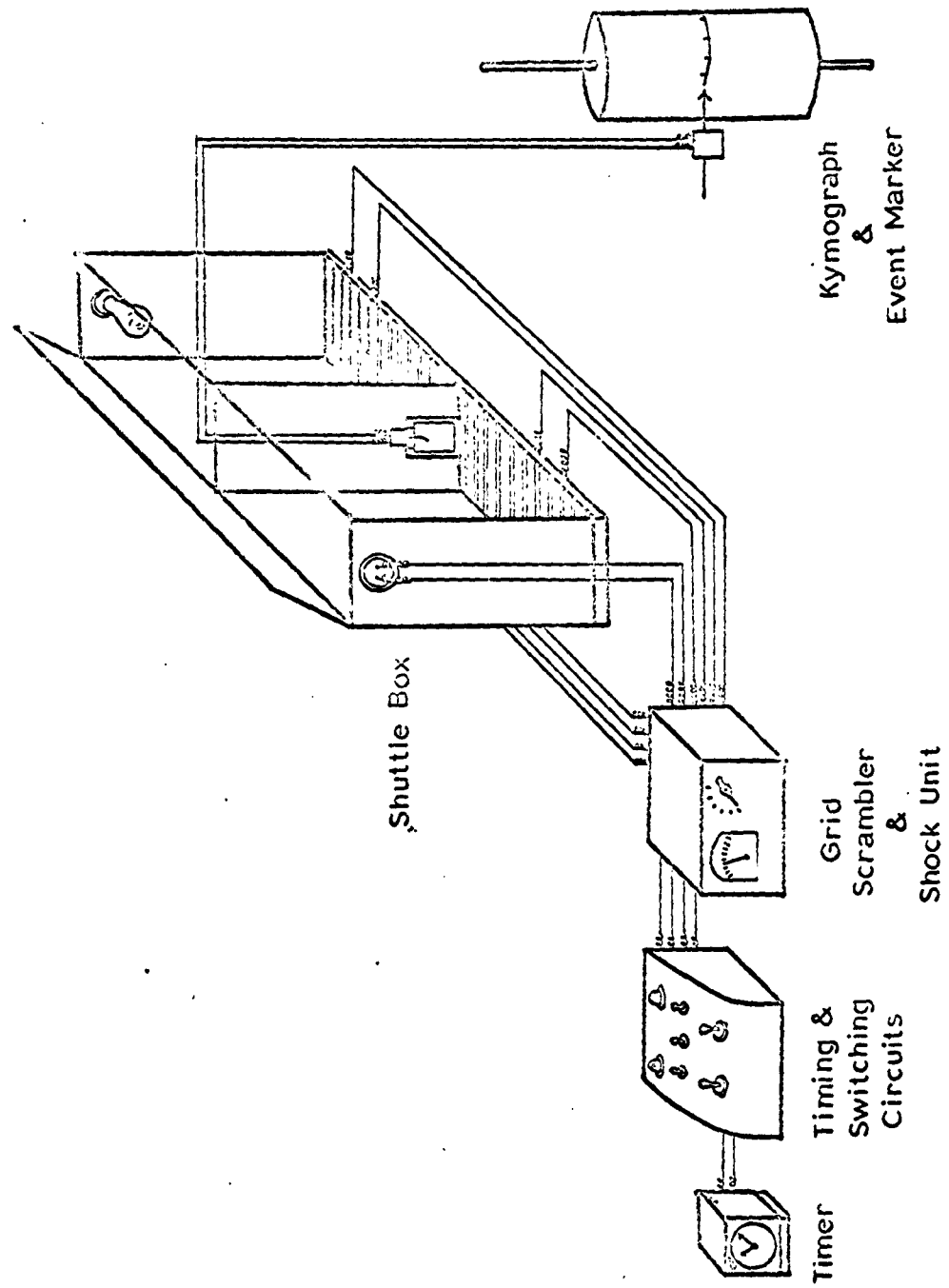Switching
Circuits

Timer

Figure 1

"crossing" kymographically. A 15 watt incandescent bulb was mounted at the top end of each compartment.

This two compartment enclosure rested on a grid constructed of brass rods 3 mm. in diameter mounted on a plastic frame in such a manner that they were separated by a space of 4 mm. and electrically isolated from each other. The grid was wired so that each of the compartments contained two separate circuits connected in series through a high resistance to a potential source capable of producing 1 to 5 milliamps current flow. An electronically operated relay switched the current back and forth between these two circuits approximately 4 times per second. This simple "grid scrambler" system in most cases effectively prevented the animal from escaping the shock either as a result of fecal pellets shorting out two adjacent bars or by learning the "pattern" of which elements carried current and which did not.

A system of cam operated microswitches programed the sequence of stimuli. These could be overridden by manual switches if desired.

D. **PROCEDURE**

A total of 14 male swiss-albino mice weighing between 20 and 28 grams were selected from a colony for the rapidity with which they mastered avoidance training. These animals were given two weeks of acquisition training of 10 trials per day. At the end of this period all were performing at least 8 avoidance responses out of the 10 trials.

The conditional stimulus (CS) was a 15 watt incandescent bulb.

Five seconds following the onset of the CS the unconditional stimulus (US) was presented. This consisted of a 1 to 5 milliampere shock delivered to the grid through the high resistance "scrambler" system. Both the CS and US remained on until the mouse escaped into the opposite compartment. In the event of a successful avoidance response the CS was terminated when the mouse crossed through the door in the center partition. The intertrial interval was one minute.

The mice were divided into experimental and control groups of 7 animals each. An attempt was made to match the members of each group as to percentage of avoidance responses, reaction time and the number of spontaneous crossings exhibited during their two week acquisition training. Seven additional days of acquisition training were given and then on the 8th to 13th days extinction training occurred. During the extinction period the US was no longer presented if the animal failed to make an avoidance response and the CS was terminated in 5 seconds.

On the 8th, 9th and 10th days of this 13 day period (corresponding to days 1, 2 and 3 of the extinction training) the members of the experimental group were injected intraperitoneally with the test compound 30 minutes prior to that day's session. The animals comprising the control group received injections of isotonic saline.

The mice (and the experimenter) were allowed to rest on the 14th day. On the following day acquisition trials were again instituted and the 13 day cycle was repeated.

The initial concept for this series of experiments was to continue testing the original 14 mice with repeated 13 day cycles.

One effective and one ineffective dose (the former 100% greater than the latter) for disrupting the conditioned avoidance response (CAR) was to be established for each of the 6 test compounds. A disruption of the CAR by a particular drug was said to occur when the correct number of avoidance responses for the experimental group was significantly below those made by the control group during the 3 days of drug or saline injection.

As testing proceeded, however, an unforeseen conflict situation inherent in the design of the experiment resulted in the loss of 4 of the original 14 mice when their level of correct avoidance responses fell below usable limits. This situation was probably engendered by the fact that during extinction training the mice were no longer shocked for failure to cross to the opposite compartment prior to 5 seconds after onset of the CS. In this situation punishment was, in a sense, "avoided" even though no crossing was made; this positively reinforced a behavioral pattern for which punishment had originally been given and a conflict resulted. One additional mouse was lost when death resulted from an injection of one of the compounds.

At the completion of the first series of compound injections, in which all 6 compounds were tested at approximately 25% of their LD_{50}, only 9 mice remained out of the original 14 animals. Because of this situation the design of the experiment had to be changed at this point. The 5 mice showing the consistently greatest number of correct avoidance responses were now assigned permanently to the "experimental" group and the remaining 4 animals served as controls.

E. RESULTS

The effect of various doses of the 6 test compounds on the
avoidance-escape situation for mice is graphically presented in
figure 2. Inspection of the individual graphs comprising this figure
reveals that during the 3 days of test injections there was, in most
cases, a definite decrease in the number of correct avoidance re-
sponses in the experimental group. The performance usually returned
to near pre-compound levels on the following three days. In con-
trast, the control group either showed little change in pre-drug
levels or various degrees of decline in the percentage of avoidance
responses during the entire six day extinction period.

The first series of experiments in which the test compounds
were injected at 25% of the LD_{50} were performed with "matched" con-
trol and experimental groups, insofar as was possible. The data was
therefore tested for significance by comparing the mean responses of
the controls to those of the experimental group for the three days on
which injections were given. This data is summarized in table 3.

From this point the control and experimental animals were no
longer varied and, as previously mentioned, 4 mice served permanently
as the control group and 5 mice permanently as the experimental group.
The results of these 13 experiments (columns 2, 3 and 4 in figure 2)
were tested for significant variation by comparing the mean response
of the 5 experimental animals during the 3 days when injections were
given to the mean response of the same animals on the succeeding 3
days. The statistical analysis was made in terms of "paired" data
and the results are summarized in table 4.
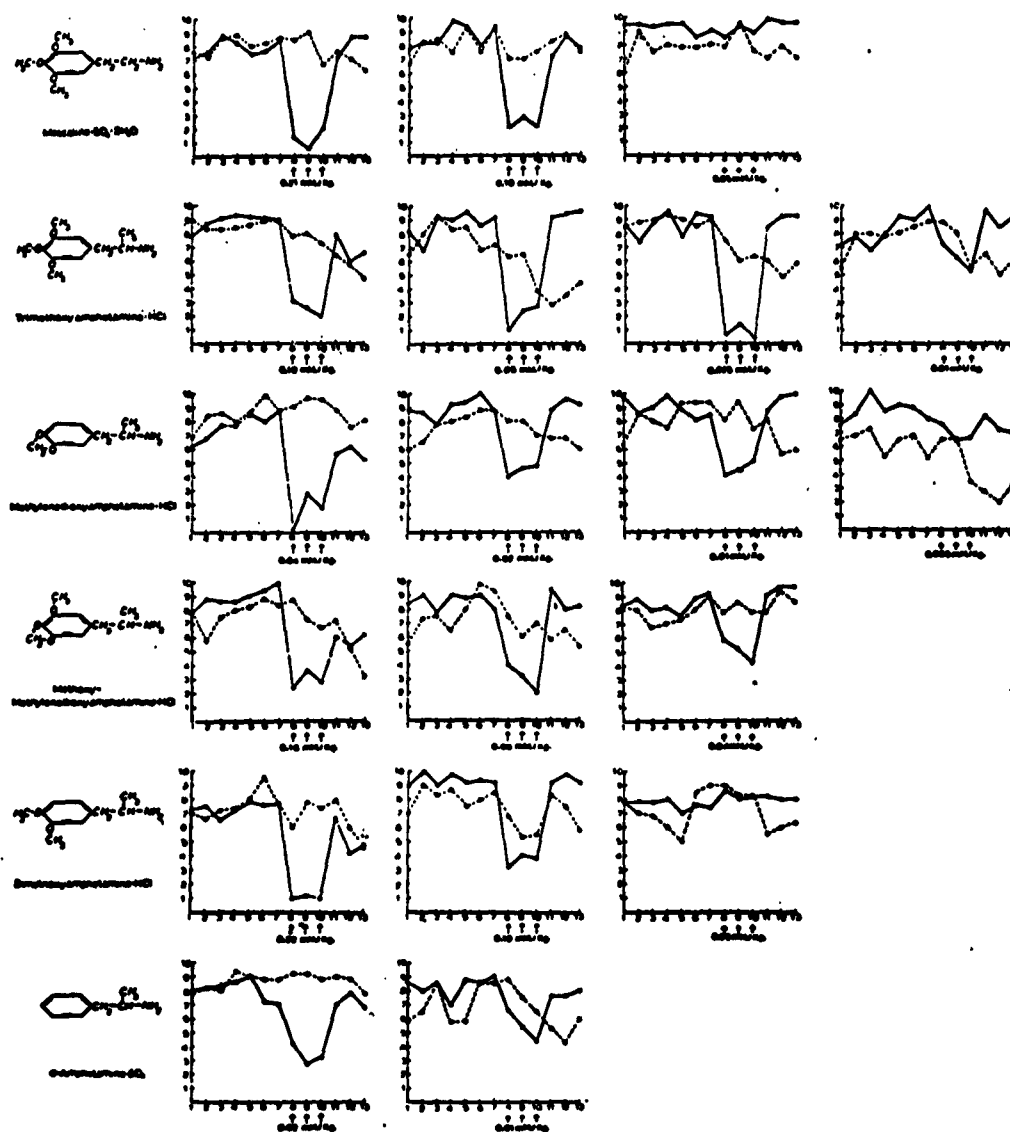
The ordinate for each graph represents the mean number of correct avoidance responses. The abcissa represents days of testing.

Arrows at days 7, 8, and 9 signify drug injections at the dose indicated.

Experimental mice are represented by open circles and solid lines; control animals by filled circles and broken lines.

Acquisition training was in force on days 1 through 6; extinction training on days 7 through 13.

Figure 2

TABLE 3

Comparing the mean number of correct avoidance
responses for control and compound-injected mice
during the first 3 days of extinction training.

(10 trials/day)

COMPOUND	DOSE mM/kg	CONTROL MEAN	EXPTL. MEAN	D.F.	"t"	P
AMPHETAMINE	.02	27.2	10.6	8	3.009	.02
MESCALINE	.21	24.2	4.4	8	5.668	.01
MDA	.04	29.4	4.8	11	9.220	.01
TMA	.10	26.0	7.7	12	3.840	.01
MMDA	.16	27.5	8.8	9	3.607	.01
DMA	.22	21.2	2.8	9	3.440	.01

TABLE 4

Comparing the number of correct avoidance responses in mice on
the first 3 days of extinction training, during which the test
compounds were administered, to the number of correct avoidance
responses on the three succeeding test days.

(n = 5)   (10 trials/day)

COMPOUND	DOSE mM/kg	MEAN DIFF.	STANDARD ERROR OF MEAN DIFF.	"t"	P(d.f=4)
AMPHETAMINE	.010	4.8	3.910	1.739	not significant
MESCALINE	.100	16.6	4.010	4.130	.02
	.050	not significant by inspection			
MDA	.020	14.2	4.483	3.168	.05
	.010	14.8	4.669	3.169	.05
	.005	not significant by inspection			
TMA	.050	20.6	3.090	6.660	.01
	.025	24.4	1.473	16.564	.01
	.010	8.6	3.592	2.394	not significant
MMDA	.080	16.4	3.316	4.945	.01
	.040	12.8	6.671	1.918	not significant
DMA	.100	17.2	4.278	4.020	.02
	.050	not significant by inspection			

The first group of experiments in which the test compounds were injected at 25% of their LD$_{50}$ showed highly significant decreases in the mean number of correct avoidance responses made by the members of the experimental group as compared to those made by the controls.

In the second group of tests (second column, figure 2) the compounds were reinjected at one-half the original dose. As shown in table 4, only amphetamine failed to produce significant results.

As mentioned above, these and all subsequent experiments analyzed the responses of the experimental group during extinction training by comparing their performance during the three days of drug injection to the succeeding three day period.

The third round of experiments (third column, figure 2) saw mescaline and the four amphetamine derivatives administered at one-half the preceding dose. Only TMA and MDA produced significant effects at this level.

In the final tests (last column, figure 2) TMA and MDA were again injected at a dose reduced by 50%. Neither compound elicited significant changes in the mean number of correct avoidance responses.

F. CONCLUSIONS

The fact that experiments are available for all compounds at doses producing both significant and non-significant effects permits a rough quantitative estimation of their relative potency in terms of disrupting the avoidance behavior of mice during extinction training.

As can be seen by inspection of table 5, MDA is the most
potent compound in this regard. Among the amphetamine derivatives,
TMA was ranked second and proved to be approximately 2 1/2 times less
potent than MDA, while MMDA was 8 and DMA 10 times less potent than
the most effective drug.

Among the two reference compounds amphetamine disrupted the
CAR at a dose comparable to that for TMA; mescaline was less effec-
tive than amphetamine and was similar in potency to DMA.

These results indicate that the amphetamine derivatives dis-
rupt avoidance conditioning in mice in an order which is roughly
similar to their relative potency for producing hallucinations in man.
The two compounds MDA and TMA are significantly more potent than DMA
in both situations. MMDA was slightly more active than DMA in mice
but unfortunately no conclusive information is available concerning
the hallucinogenic potency of the former compound in man. As previ-
ously reported, Alles (1962), experimenting on himself, judged MMDA
to be 2 to 3 times less potent than TMA for producing threshold sub-
jective effects and in mice the former compound proved to be 4 times
less potent than the latter.

Of the two reference compounds, the results obtained with mes-
caline in mice apparently relate to the human situation since this
compound is significantly less potent than either MDA or TMA in both
situations. Reduction of avoidance behavior in mice failed, however,
to differentiate amphetamine from the other test compounds. Ampheta-
mine is not considered to be primarily a hallucinogenic agent in man
and yet in mice it exhibited an activity comparable to that of TMA

TABLE 5

Comparing doses of the test compounds which produced
significant decreases in avoidance behavior in mice
with doses producing no significant changes.

DOSE (mM/kg) Producing:

Compound	Significant Decrease	No Significant Change
MDA	.010	.005
TMA	.025	.010
MMDA	.080	.040
DMA	.100	.050
AMPHETAMINE	.020	.010
MESCALINE	.100	.050

which is a potent hallucinogenic agent in man.

A close correlation apparently exists between the toxicity of the amphetamine derivatives and their effects on avoidance conditioning. A comparison of table 1 with table 5 will reveal a very similar ranking as given both by the LD_{50} values and the relative doses of these compounds necessary for disrupting the conditioned response. The spread in the range of the toxic doses is not so marked as in the avoidance situation but the amphetamine derivatives fall into approximately the same relative order for both tests. The results for mescaline also agree rather closely. Amphetamine proved to be the most toxic compound in mice but had only one-half the potency of MDA for disrupting avoidance behavior; however, its LD_{50} value was calculated for animals housed in groups of 4, rather than singly, following injection. Chance (1946) has shown that the toxicity of amphetamine is markedly increased by aggregation of the test animals. If our test had been conducted on singly housed instead of aggregated mice, amphetamine would no doubt have proved to be somewhat less toxic and thus could conceivably have shown a better correlation between its toxicity and its ability to disrupt avoidance conditioning.

During the test runs the mice often "spontaneously" crossed from one compartment to the other prior to onset of the CS and while the number of such crossings was recorded for each experiment the data did not yield any significant information with regard to drug effect. It was known that several of the test compounds were capable of producing potent locomotor stimulation in mice (Fairchild, 1962) and it was hoped that the crossing rates would reflect such stimula-

tion. Each mouse, however, reacted to the test compounds in a fashion characteristic for him by either showing an increased or a decreased rate of spontaneous crossing during the three days of compound injection.

G. DISCUSSION

Numerous publications in the literature report the effects of hallucinogenic agents on animal behavior. A large number of these papers are concerned with the activity of psychomimetic compounds other than mescaline, especially LSD-25. While the importance of this information is recognized it will not be discussed. Since the investigations we performed in mice were concerned with the activity of amphetamine derivatives, which are mescaline-like compounds, only those reports dealing specifically with mescaline will be considered. No attempt will be made to generalize the results to include hallucinogenic agents as a whole.

There are essentially two types of observations made concerning the effects of drugs on animal behavior; in the first the compound is administered to an animal and changes in the "spontaneous" behavior are noted; in the second type the animal is conditioned to perform a specific "learned" behavioral response and drug effects are judged by alteration of this performance.

Under the first type of observation the following papers can be considered. Witt (1951) reported that mescaline decreased and LSD-25 increased the accuracy with which a spider formed its web. Sturtevant and Drill (1956) injected mescaline intraventricularly

into cats and dogs and observed a number of autonomic effects which preceded the development of long lasting catatonic states. Maffii and Soncin (1958) described a stereotyped "ear scratching" produced by mescaline in mice and showed that its incidence was dose related. Deegan and Cook (1958) reported that various tranquillising agents antagonized this scratching phenomena. Haley (1957) injected mescaline and LSD "intracerebrally" into conscious mice; the former drug produced aggressive behavior and paroxysms of ear scratching followed by depression while the latter compound resulted in initial hyperexcitability followed by long lasting stupor.

In addition to the publications mentioning changes in "spontaneous" behavior there are a number of reports in the literature concerning the effects of mescaline on "learned" behavioral patterns. Sivadjian (1934) injected 100 mg/kg of mescaline into a rat trained to avoid a shock by leaping a barrier. After receiving the drug this animal was reported to have reacted to a tone just as if it were the shock itself and experienced what Sivadjian termed "a hallucinatory crisis." Courvoisier (1956) confirmed this observation in Norwegian Pibald rats which, according to this author, are quite sensitive to mescaline; following intramuscular injections of mescaline (neither salt nor dose stated) these animals stopped climbing a rope to avoid shock and reacted to the tone (CS) with a "veritable dementia" consisting of squealing, leaping up and down, biting the grid bars, etc. Unconditioned rats showed only increased irritability to the tone. Bridger and Gantt (1956) studying a classically conditioned leg flexion in dogs reported that 70 mg/kg of mescaline injected intra-

muscularly essentially eliminated this conditioned response; the drug also caused these animals to bark or howl on presentation of the tone (CS), a behavior they normally exhibited only to the shock (US) itself. These observations are similar to those described by Sivadjian (1934) and Courvoisier (1956) for the rat and seem to indicate that mescaline somehow increases the potency of the CS to the point where it begins to take on properties usually associated only with the US. Somewhat similar observations were made by us in mice. Locomotor depression was commonly observed in unconditioned animals following mescaline injection. Conversely, mescaline usually produced locomotor stimulation in mice conditioned to avoidance behavior. This apparent stimulation was more or less constant during the time the animals were in the behavioral apparatus and did not normally show a sharp increase on presentation of the CS. In this latter respect, our observations differed somewhat from those discussed above.

Maffii (1959), using an apparatus originally described by Cook, Weidley, Morris and Mattis (1955), conditioned rats to avoid a shock by leaping onto a vertical pole. A tone was used as the CS. According to this investigator, after the avoidance response had been established, additional training often resulted in the formation of what was termed a "secondary conditioned response" which was characterized by a jumping reaction prior to presentation of the CS. Mescaline injected intraperitoneally at 33 mg/kg was reported to inhibit selectively the secondary conditioned response without affecting either the primary avoidance or the unconditioned response. This study is difficult to interpret because Maffii's secondary condition-

·1 response is not clearly defined. He did not describe the nature of the intertrial interval employed but if it were constant the so-called secondary conditioned response could actually be temporal conditioning with the animals responding to a fixed time interval between shocks. A constant intertrial interval of one minute, in our experiments, produced temporal conditioning in a number of mice. However, mescaline sulfate at .10 mM/kg (approx. 30 mg/kg) did not uniformly decrease intertrial response rates (in some instances increases were observed), although it significantly depressed avoidance behavior at this dose. To the extent we understand the terms used by Maffii our results would seem to indicate that the interaction of mescaline with intertrial responses and avoidance behavior is not as clear cut in the mouse as he indicated for the rat, where one response could be selectively eliminated without effecting the other.

Among the publications concerning the effects of mescaline on "learned" responses we were fortunate enough to discover the doctoral thesis of S. L. Chorover (1959). This investigator described four experiments in which mescaline was shown to inhibit conditioned behavior. Among these four tests the drug caused its most pronounced effect when given during the extinction of a conditioned avoidance response and for this reason we decided to pattern our experiments along these lines. Chorover conditioned rats in an avoidance situation using a two compartment shuttle box. The CS was a buzzer and the US an electric shock and the animals were run until they reached a criterion of 90% correct avoidance responses. Extinction training was then instituted during which failure to respond to the CS was no

longer punished by shock. One group of rats received intraperitoneal injections of 25 mg/kg mescaline sulfate (.09 mM/kg) on the first 4 days of extinction training. These animals showed an immediate and complete extinction of the avoidance response on the first day of drug injection. The percentage of avoidance behavior remained approximately zero for the next 3 days of mescaline administration and also during the succeeding 11 days of testing during which no drug was given. A control group, injected with normal saline, showed a typical extinction pattern with a gradual decrease in the number of avoidance responses during the 15 days of extinction training.

Our experimental situation differed from Chorover's in several major respects; for instance instead of rats we employed mice; there were several reasons for this. In the first place complete toxicity data in this species was available for the test compounds and secondly approximately 10 times as much of these compounds, on a per kilogram body-weight basis, would have been necessary if rats were used. This latter point was of importance since some of the compounds in the series represented much labor by Dr. Alles and his staff and it was desirable to use them as sparingly as possible. Because of the well known effects of mescaline on the visual system we decided to use a visual rather than an auditory CS. Our period of compound injection was 3 rather than 4 days. Since we were primarily interested in using behavior to compare the effects of compounds rather than employing the compounds to investigate behavior, per se, our period of extinction training was shortened from 15 to 6 days.

In spite of differences in both procedural matters and animal

species a degree of similarity still existed between the results obtained by Chorover and those obtained by us. In both situations mescaline injected at approximately .10 mM/kg markedly depressed conditioned avoidance behavior during extinction training; however, rats, as tested by Chorover, seemed a good deal more sensitive to the drug than did the mice used in our experiments. As mentioned above, the rat exhibited an almost complete loss of the conditioned response which extended well past the period of mescaline injection; mice, on the other hand, although showing significant depression of avoidance behavior during the 3 days of compound injection exhibited almost complete recovery on the following 3 days.

In addition to the experiments where extinction training was started simultaneously with administration of mescaline, Chorover ran another group of animals in which extinction was not started until after the 4 days of injection had been completed; these rats were simply returned to their cages during this period after being injected with 25 mg/kg of mescaline. Their extinction rate was almost identical to that of the control group. Chorover interpreted these results as indicating that the interaction of mescaline administration and extinction training was necessary in order to produce suppression of avoidance behavior in the rat and concluded that the persistence of this effect beyond the period of injection was not residual drug activity. He states that, "the animals are capable of transferring the 'new rules of the game' (i.e. that the US no longer follows the CS) to the post-drug training." If such an interpretation were true, we would have to conclude that the mice are evidently not cap-

able of making such a "transfer" since in our experiments, although the test compounds caused significant depression of the CR, in mice there was almost complete recovery of the response on the first day following the period of drug injection.

The major difficulty encountered during our behavioral experiments has previously been mentioned (part D of this chapter). An unforeseen conflict situation inherent in the design of the experiment weakened the conditioned response in a number of mice to the point where they had to be eliminated as experimental subjects. This seriously reduced the number of animals participating in the test and necessitated a change in procedure during the course of the investigation. While statistically significant differences could be obtained under these conditions the situation was certainly marginal; had one additional animal been lost from the experimental group the tests probably could not have been completed. It seems evident that repeated cycles of acquisition and extinction training in mice, at least under conditions of our experiment, are not a satisfactory method for comparing the activity of a series of drugs on avoidance behavior.

H. SUMMARY

The test compounds decreased the number of correct avoidance responses during extinction training in mice conditioned to avoid a shock by crossing from the illuminated to the nonilluminated compartment of a shuttle-box. MDA proved to be the most potent compound in this regard among the 4 amphetamine derivatives; TMA had 50%, MMDA

had 12% and DMA had 10% of its activity. This order of potency has a rough correlation with the ability of these compounds to produce hallucinatory phenomena in man.

In considering the reference compounds, mescaline seemed to agree properly in both the human and the mouse, but amphetamine did not. The latter drug, while markedly disrupting avoidance behavior in mice, does not normally produce hallucinations in man.

The order in which the test compounds disrupt avoidance behavior in mice is very similar to the order of their toxicity in the same species.

CHAPTER IV

THE EFFECTS OF THE AMPHETAMINE DERIVATIVES

ON SPONTANEOUS BRAIN ELECTRICAL ACTIVITY

IN THE CONSCIOUS CAT

A. INTRODUCTION

As previously mentioned in Chapter II, mescaline and the amphetamine derivatives exhibited the property of reducing the amount of ongoing brain electrical activity in the barbitalized cat following close intracarotid injection. In addition, TMA and MDA were more potent than DMA and mescaline in this regard. This fact interested us since the order of activity was somewhat analogous to the hallucinogenic potency of these compounds in man.

Prior to pursuing the effects of the test compounds on spontaneous brain electrical activity in the anesthetized cat, we became aware of the work of Adey and Dunlop (1960) who analyzed changes in behavior and in the electro-encephalogram (EEG) of conscious cats induced by two cyclohexamine derivatives; they were 1-(phenylcyclohexyl) piperidine monohydrochloride (CL-395, Sernyl) and n-ethyl-1-phenyl cyclohexylamine monohydrochloride (CL-400, cyclohexamine). These compounds were originally tested and used as analgesic agents in major surgery but are rarely employed today because of the marked mental disturbances they produce; hallucinations along with delirium and odd behavioral patterns have been reported (Lear, Suntay, Pallin and Chiron, 1959). In the conscious cat these compounds resulted in disruption of approach behavior in a T-maze which existed simultane-

66

ously with marked alterations in the electrical activity of various deep structures in the brain. These electrical patterns consisted of fast activity alternating with irregular slow waves and seizure discharges and were centered in the hippocampal system.

The interesting correlations published by Adey and Dunlop (1960) between alterations in brain electrical activity and simultaneous disruptions of behavior produced by the cyclohexamine type hallucinogenic agent intrigued us since our test compounds had also caused EEG changes in the cat and were able to disrupt avoidance behavior in the mouse. (Chapters II and III.)

Although we were not prepared to train cats in a T-maze, it was decided to analyze the effects of the test compounds in the conscious animal to determine if alterations in the electrical activity of deep structures, similar to those produced by the cyclohexamines, would occur; hopefully, such an alteration might reflect quantitative differences between various members of the drug series.

With the kind help of Dr. Douglas Stuart (Veterans Administration Hospital, Long Beach, California) the necessary techniques were mastered for making preparations of this kind and 8 cats were subsequently prepared with chronically indwelling electrodes implanted in various deep structures of the brain. Cortical recordings were also taken from electrodes within, but not through, the skull.

In general, it was subsequently demonstrated that the amphetamine derivatives and one of the reference compounds, mescaline, did indeed produce marked changes in the ongoing electrical activity in the brain of the conscious cat. These changes were in the nature of

an increase in amplitude and decrease in frequency as compared to the predrug wave forms; the high amplitude, slow waves often appeared as hypersynchronous bursts at frequencies between 2 and 10 cycles per second which alternated with low voltage, fast activity. Figure 3 illustrates these patterns for three of the test compounds in one cat. Alterations in brain electrical activity coexisted with what appeared to be a highly alert, and paradoxically, immobile state in the animal.

The other reference compound, amphetamine, also produced a high degree of behavioral alerting but had just the opposite effects of mescaline and the amphetamine derivatives in that it caused marked and unfluctuating increases in the frequency and reductions in the amplitude of ongoing brain electrical activity; this is the well known "desynchronizing" or "alerting" effect of amphetamine on the EEG. (Hiebel, Bonvallet, Huve and Dell, 1954; Rinaldi and Himwich, 1955a.)

In the hope of getting quantitative data concerning the effects of these drugs on brain electrical activity an 8 channel frequency analyzer was constructed; this instrument was then employed in analyzing the alterations produced by the test compounds in 6 of the 8 chronically implanted cats. Three cats were employed in making a comparison between the control EEG record taken during a period when the animals were forced to remain alert and a post-injection record in which the drug produced the alerting. Another group of three cats was conditioned to a tone paired with an air blast which was delivered to the animals via a pair of limp, rubber "flapper" tubes. In
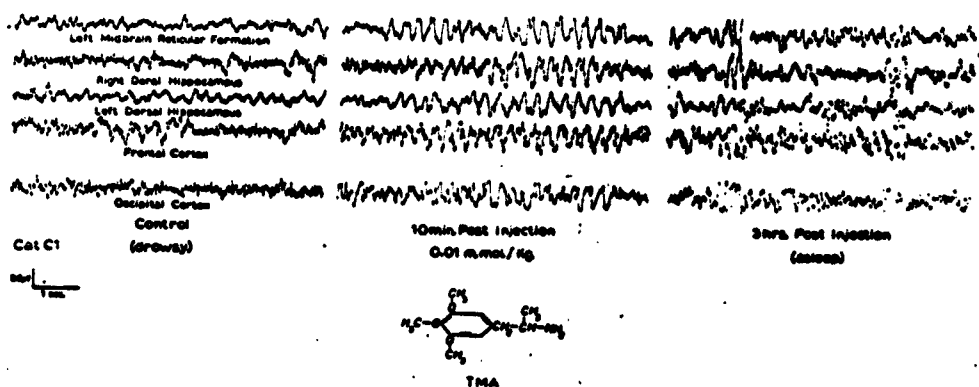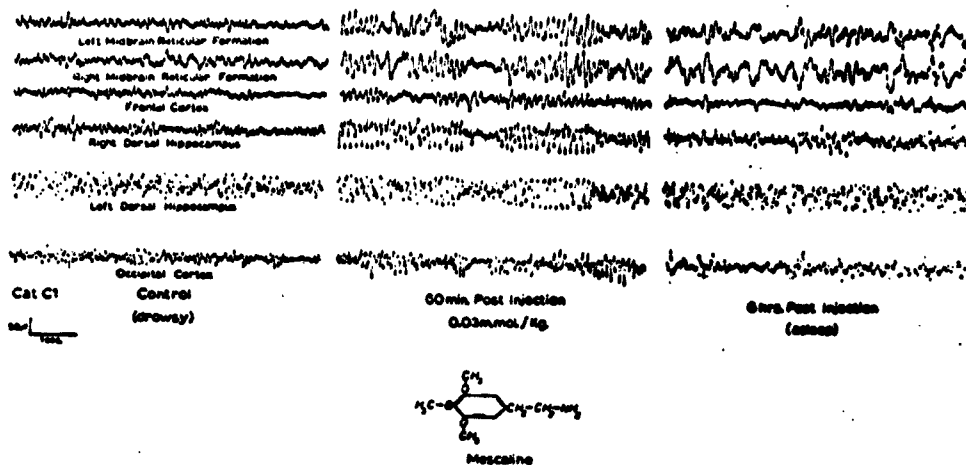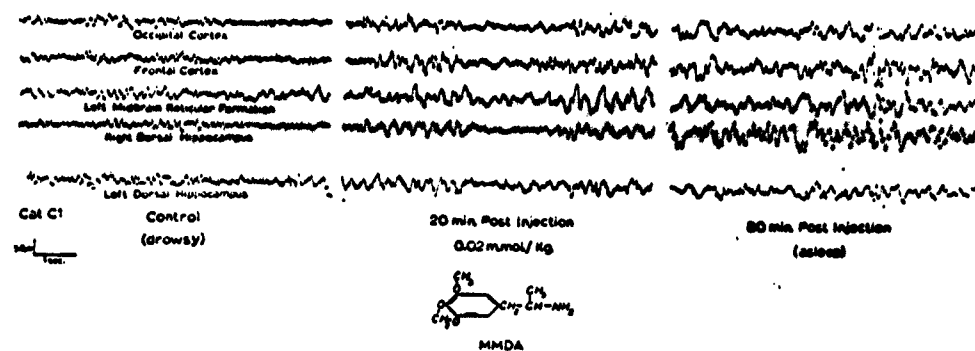
Hypersynchronous bursting activity produced in the brain of an unanesthetized and unrestrained cat by the intraperitoneal injection of mescaline and two phenyl substituted amphetamine derivatives.

Figure 3

these animals an attempt was made to analyze the effects of the test compounds upon the electrical and behavioral manifestations of the conditioned and unconditioned response and to quantitatively estimate their relative potency for altering the frequency distribution by comparing the regression coefficients on the analyzer channel numbers.

B. INSTRUMENTATION AND METHODOLOGY

1. The Frequency Analyzer

This instrument was designed for us by Dr. Donald J. Jenden of the Department of Pharmacology. Without his expert knowledge and unselfish contribution of his valuable time this phase of the investigation could not have been undertaken. The actual construction of the instrument was performed in the electronic shop of the Biophysics Department. We owe Mr. Dewig and Mr. Smith of that department a debt of gratitude for numerous, helpful technical suggestions.

The analyzer primarily consists of 8 tuned AC amplifiers coupled to appropriate rectifier and integrator circuits. Figure 4 gives the schematic diagram for these units. The resistor and capacitor valves as well as the frequency response of each channel are shown.

The input signal for the analyzer is taken from one side of the last stage of the preamplifier incorporated in the Grass Model III D electroencepholograph. This gives approximately a 250 fold amplification of the voltage

+180 VDC

INPUT

−1.34 VDC

RESET

OUTPUT

Figure 4

FIXED RESISTORS (½W, 20%)

R2	1M	R14	390K
R3	1M	R16	1M
R4	1M	R18	10M
R5	220K	R19	1M
R6	330K	R23	470K
R7	330K	R24	100K
R9	1M	R25	39K
R10	1M	R26	1M
R11	1M	R27	47K
R12	220K	R28	100K
R13	390K		

TUBES

V1 & V2	7025
V3	5726
V4	6136
V5 & V6	12AT7

FIXED RESISTORS (½W, 1.0%)

R20	60K
R22	20K
R29	100K
R31	82K

VARIABLE RESISTORS

R1	10K
R8	50K + 100K fixed
R15	50K + 120K fixed
R17	10K
R21	25K
R30	5K

CHANNEL No.	FREQUENCY RANGE (c.p.s.)
I	2.38 – 3.49
II	3.49 – 5.13
III	5.13 – 7.53
IV	7.53 – 11.03
V	11.03 – 16.20
VI	16.20 – 23.80
VII	23.80 – 34.90
VIII	34.90 – 51.30

CAPACITOR VALUES IN MICROFARADS

$C_{1,2,6}$	C_3	$C_{4,5,8}$	$C_{9,10}$	C_7	C_{11}
1.00	0.330	0.220	0.150	0.150	1.0
1.00	0.220	0.150	0.100	0.100	1.0
0.47	0.150	0.100	0.068	0.067	1.0
0.47	0.100	0.068	0.047	0.047	1.0
0.22	0.068	0.047	0.033	0.033	1.0
0.15	0.047	0.033	0.022	0.022	1.0
0.10	0.033	0.022	0.015	0.015	1.0
0.10	0.022	0.015	0.015	0.010	1.0

fluctuations seen by the electrode implanted in the cat's brain. This amplified signal is then fed into a primary amplification stage in the analyzer with a gain variable between 0 and 70 which serves as a control of signal input level. All 8 channels are in parallel with this output. Each tuned amplifier selects the AC frequency corresponding to its band pass filter, amplifies and rectifies it to DC and integrates this current for one minute. The output capacitor is then discharged through closure of a relay to a cathode follower output circuit.

The sequence of the 8 discharging relays is controlled by microswitches actuated by cams driven by a synchronized motor. Each channel is sequentially discharged to the output circuit once each minute. The write out time is approximately one second per channel. Each channel is reset by the relay which follows it in sequence so that the total integration time for each is constant.

The output of the cathode follower varies between 0 and 20 volts. A portion of this low impedance output is led through a voltage divider into an Esterline-Angus Model AW Recording DC Milliammeter.

The maximum charge on the output condensers of the tuned amplifiers can reach approximately 32 volts DC under the operating conditions employed. This was equated to approximately a full scale deflection on the recording dynograph. Each channel was then balanced by adjusting

the bias voltage on its amplifier so that at a given set-
ting of the primary amplifier an input signal of 8 milli-
volts with a frequency corresponding to the peak transmis-
sion point produced a 50% scale deflection on the dyno-
graph. The analyzer was calibrated in this fashion before
and after each experiment to insure equal amplification in
all channels over the time period involved.

A certain amount of drift in the amplifier balance
at reset or base line conditions occurred and this was
checked periodically throughout the experiment.

2. Implantation of Chronic Electrodes in the Cat

Cats of either sex weighing between 2.8 and 3.2 kg
were selected as subjects. The animal was anesthetized
with an initial dose of 35 mg/kg sodium pentobarbital.
The head was clipped, shaved clean, scrubbed with soap and
water and washed with alcohol. The cat's head was then
mounted in a Trent-Wells Stereotaxic Apparatus and a mid-
line incision was made extending in an anterior-posterior
direction from approximately the junction of the frontal
and nasal bones to the cephalic aspects of the sagittal
crest. The skin was dissected free from the underlying
muscles and pulled back. The temporalis muscles were care-
fully cut along their insertion on the medial aspects of
the parietal bone and reflected back from the skull. This
exposed most of the dorsal aspects of the parietal bone as
well as the caudal portions of the dorsal frontal bones

anterior to the coronal suture. The area was isolated with gauze packs and the periosteum was scraped from the bone. From this point on care was taken to keep the field clean and dry.

Four 2 x $\frac{1}{4}$ nickel plated wood screws were screwed into, but not through, the bone at the corners of the ex- posed field through drilled starter holes. Two of these screws had insulated 40 gauge stainless steel wires sol- dered to them. One was placed in the left frontal bone 5 or 6 mm. anterior to the coronal suture and the other on the dorsal-lateral curvature of the caudal aspect of the right parietal bone. These served as reference and ground electrodes respectively. A bridge of Caulk's NuWeld den- tal cement was then laid down to connect these four sup- port screws. This served to isolate the remainder of the field from the skin and muscle and the dry packs original- ly used in these areas were replaced with saline moistened gauze.

The stereotaxic coordinates corresponding to the desired dimensions for electrode placement had been calcu- lated according to the atlas of Jasper and Ajmone-Marsan (1960). The anterior-posterior and medio-lateral coordin- ates for the depth electrodes were then marked off on the skull surface. Placement of skull electrodes were also demarked. Small burr holes were drilled through the skull exposing the underlying dura in those areas in which depth

electrodes were to be placed. Small starter holes not
penetrating through the bone were made at those sites cor-
responding to the desired skull electrodes. The dura ex-
posed through the burr holes was carefully sectioned.
Subsequent bleeding and loss of cerebrospinal fluid were
controlled, using bone wax if necessary, and the field was
again carefully cleaned and dried.

Depth electrodes were bipolar. They consisted of
two insulated 40 gauge stainless steel wires affixed to a
section of stainless steel tubing insulated by 6 to 8
coats of Epoxylite 6001-m electrode insulator which had
been successively baked on in an oven maintained over 140°
C. The wires were cut 2 mm. below the central shaft and
these sectioned ends represented the only electrically ex-
posed surface. The distance between the tips was approx-
imately 1 mm.

These electrodes were stereotaxically positioned
over the proper burr hole and carefully lowered past the
sectioned dura to the proper depth within the brain.
Bleeding was controlled when necessary by small pieces of
Gelfoam packed into the burr hole. The area was again
carefully dried and the electrode secured in position with
dental cement. When the cement had dried sufficiently,
the electrode carrier was carefully removed, the wires
were stripped away from the central shaft which was then
sectioned close to the skull. This procedure was repeated

until all deep electrodes had been positioned.

Cortical or skull electrodes consisted of 0 x $\frac{1}{4}$ round head, nickel plated wood screws to which a length of insulated 40 gauge stainless steel wire had been soldered. These were screwed into the proper starter holes and covered with dental cement.

The wires leading from the various electrodes were now soldered into two nine pin Winchester MGS octagonal plugs. These plugs were positioned one behind the other some 5 mm. apart in the midline and the superstructure of the implant was built up around them by successive layers of dental cement. After sufficient time had been allowed for drying, all edges of the implant which would come into contact with tissue were carefully polished with the drill to eliminate any rough spots. The skin was closed around the implant after carefully flushing the field with saline and exploring for foreign matter which might have become lodged between the temporal muscles and the skin or skull during the course of the operation.

Careful post-operative nursing care was given the animals since the procedure was relatively lengthy and considerable central trauma was no doubt sustained. Most animals fared well and only two cats expired out of ten attempted. One was lost during the course of the operation and the other died shortly after it had been removed from the stereotaxic apparatus.

C. COMPARING PRE-DRUG AND POST-INJECTION ALERT STATES

1. Procedure

Three female cats with chronically implanted elec-
trodes were used in these experiments. They were housed
in a Faraday cage during the recording sessions; this
screened enclosure was covered on the top and three sides
by black plastic sheeting and was brightly illuminated
from above. The laboratory was maintained dark. Such an
arrangement permitted easy observation of the animal and
these conditions limited the visual field of the cat and
to some extent directed its attention toward the experi-
menter.

The sockets on the animal's head were connected by
cables to a Grass Model III D Electroencephalograph and
through one of the preamplifiers of this instrument to the
frequency analyzer. The cat was isolated from cage ground
by a glass plate occupying the entire floor of the Faraday
cage and was connected to a machine ground through its
right temporal electrode.

Following calibration of the analyzer and electro-
encephalograph quiet was maintained in the laboratory
until 30 minutes of analyzer record had been taken during
which the cat was obviously "drowsy" or "asleep." The
animal was then alerted by illuminating the laboratory and
allowing it a clear view of the experimenter. The alert
state was maintained while another 30 minutes of analyzer

record was obtained. In addition to the continuous analyzer record, which gave minute to minute integrated patterns of one intracerebral electrode, 6 channels of standard EEG recordings were periodically taken from various deep and surface sites.

Following the two "control" periods the cat was injected intraperitoneally with a dose of one of the test compounds dissolved in 2 ml. of distilled water. Two 30 minute post-injection records were then taken. The first of these ran from 30 to 60 minutes post-injection or started from that point in time at which the analyzer record exhibited obvious changes. The second record was usually obtained several hours later and, if possible, at a time when drug induced changes appeared to be abating. Drug induced physiological and behavioral changes were recorded as observed.

2. Electrode Placements

Cat CI-2

Female, short-haired, steel blue weighing 3.0 kg. The electrodes were calculated to be in the following locations (Jasper and Ajmone-Marsan, 1960):

AREA	STEREOTAXIC COORDINATES		
Right Midbrain Reticular Formation	P+ 3.0	L3	H-1.0
Right Dorsal Hippocampus	P+ 4.0	L6	H+7.0
Left Dorsal Hippocampus	P+ 4.5	L5	H+7.5

Cortical electrodes were placed bilaterally in the frontal

and occipital areas.

Cat CI-4

Female, short haired calico weighing 3.0 kg.

AREA	STEREOTAXIC COORDINATES		
Right Midbrain Reticular Formation	P+ 3.0	L3	H-1.0
Left Dorsal Hippocampus	P+ 4.5	L5	H+7.5
Right n. Medialis Dorsalis of the Thalamus	P+ 7.5	L2	H+3.5
Left Amygdaloid Area	P+ 14.0	L10	H-6.0
Left Septal Area	P+ 14.5	L1.8	H 4.5

Cortical electrodes were placed bilaterally in the frontal, parietal and occipital areas.

Cat CI-5

Female, long haired calico weighing 2.8 kg.

AREA	STEREOTAXIC COORDINATES		
Right Midbrain Reticular Formation	P+ 3.0	L3	H-1.0
Left Dorsal Hippocampus	P+ 4.5	L5	H+7.5
Right Dorsal Hippocampus	P+ 4.5	L5	H+7.5
Left Septal Area	P+ 13.0	L1.8	H+4.5

Cortical electrodes were placed bilaterally in the frontal, parietal and occipital areas.

A histological check of the subcortical electrode placements has not been completed. The frequency shift toward slow wave, high amplitude activity was so generalized throughout all recording electrodes (see figure 3) that precise location is not essential for the present
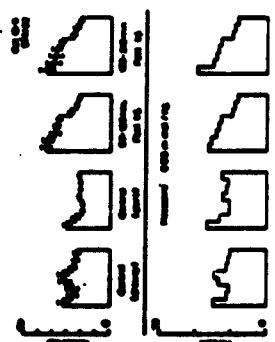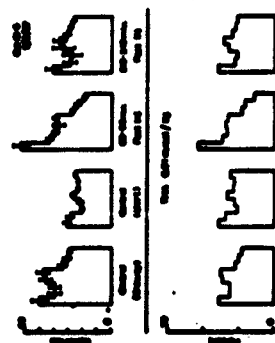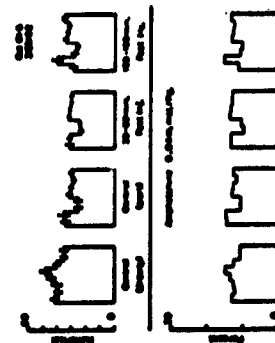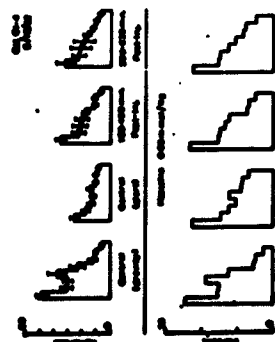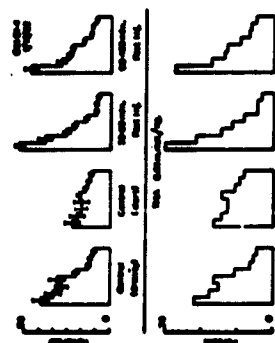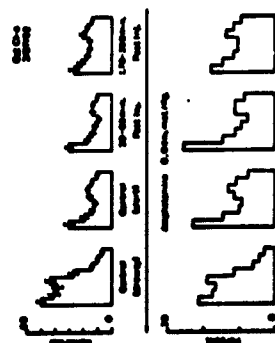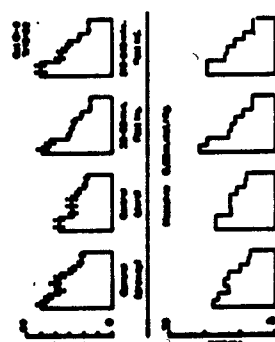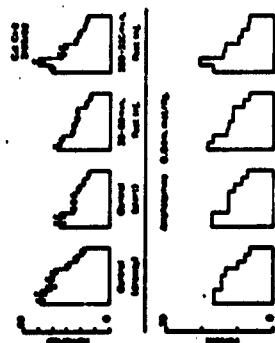
arguments.

3. **Results**

a) General Considerations

A number of parameters can be observed during
experiments of this kind. Since the animals are un-
anesthetized and essentially unrestrained a host of
gross behavioral and physiological changes result from
administration of the four amphetamine derivatives and
the two reference compounds, mescaline and amphetamine.
Some estimation as to the degree of these effects
could be made and on this basis relative drug poten-
cies might be assigned. Quantification of such gener-
al information poses a difficult problem, especially
when dealing with a small group of animals and for
this reason, with but one exception, no attempt will
be made to do so. This exception involves the state
of "alertness" produced by the test compounds which
has been given an arbitrary importance in the quanti-
tative comparison of a more objective measurement, the
frequency analysis of the ongoing brain electrical
activity. In order to compare adequately the pre-drug
and post-injection frequency analysis it was desirable
to select those conditions in which the control rec-
ords showed the greatest degree of consistency; the
alerted animal was deemed satisfactory from this
standpoint since the minute to minute analyzer pat-

terns showed striking similarity in this state. Figure 5, among other things, depicts a series of "alert" control analyzer patterns for each of the 3 cats used in this experiment. It will be noted that the standard deviation of the frequency distribution is comparatively low during this period and that this pattern is generally similar from day to day for any one cat. In contrast, the "drowsy" control analyzer patterns show a larger standard deviation and a greater daily variation in frequency distribution. This is understandable since a constant state of alertness can easily be maintained by manipulation of the animal's environment but the degree of drowsiness is largely up to the cat.

Another important reason exists for choosing the alert state for control conditions; as previously mentioned, mescaline and the four amphetamine derivatives although eliciting highly alert behavioral states, characteristically produce low frequency, high voltage waves in the brain electrical activity. Similar changes occur during normal "non-alert" states and in general these become more pronounced as the animal goes from light "drowsiness" to sleep. Occurrence of such phenomena in the control records would, of course, interfere with the analysis of the drug induced states.

From the above considerations it is apparent
that low frequency, high voltage activity could appear
in the post-injection record as the result of either
drug activity or normal drowsiness. In order to avoid
the latter contingency it was decided to eliminate
those experiments in which any apparent "drowsiness"
occurred during the post-injection period. Only those
doses of the test compounds which produced obvious
alerting were considered usable for purposes of quan-
titative comparison. While this point was arbitrary,
the stereotyped behavior produced by "effective" doses
of the test compounds was so characteristic that mak-
ing the decision "alert" or "drowsy" was usually sim-
ple. With few exceptions the cats which received an
effective dose adopted a prone position with their
head up and all four limbs extended stiffly to one
side. The facial expression was usually one of in-
tense alertness and occasionally the animal would open
its jaws and appear to hiss or pant. Often the cat
would stare slowly about the Faraday cage and then
suddenly fix its attention on some particular portion
of the environment which it would regard with particu-
lar intensity for a number of moments; during this
time it was not uncommon for the cat to hiss and with-
draw its head in a fashion somewhat akin to a defen-
sive maneuver. In a few instances animals were ob-

served to actually extend an exploratory paw toward
that portion of their environment which apparently
interested them so highly. These phenomena occurred
in surroundings with which the cats were thoroughly
familiar and in which non-drugged animals usually con-
ducted only brief explorations. Could these animals
be experiencing hallucinations? The desire to answer
this question in the affirmative is very strong when
one has actually observed the above patterns of be-
havior.

Along with this relatively immobile but highly
alert state the four amphetamine derivatives and mes-
caline produced hypersynchronous bursts of low fre-
quency, high voltage activity in the brain, as pre-
viously discussed. However, amphetamine itself, while
causing marked alerting in cats did not elicit high
voltage, slow waves in the ongoing electrical activity
but, on the contrary, produced a "desynchronized" pat-
tern composed of low amplitude, fast waves.

Since amphetamine produced essentially the
opposite effects from mescaline and the mescaline-like
amphetamine derivatives, its activity in the central
nervous system was judged to be clearly different from
the former compounds and it was no longer considered
from the standpoint of quantitative comparisons of
potency in these particular tests.

b) Quantitative Estimates of Relative Drug Potency

The dorsal hippocampus was chosen as the location in the brain from which the frequency analysis was to be made; it showed marked hypersynchronous bursting upon administration of the test compounds and was also the region which exhibited a similar type of activity following administration of cyclohexamine derivatives (Adey and Dunlop, 1960).

As Burch (1959) has pointed out, one of the greatest difficulties in working with the frequency analysis of brain electrical activity is in determining how to present the plethora of data in a concise and understandable form. While we have not solved this difficulty our approach to the problem was essentially to abandon a very large segment of the collected information and to work only with that data collected in the first 3 channels of the analyser. These channels have maximum frequency responses of approximately 3, 5, and 7 cycles per second and since this roughly corresponds to the frequency range of the hypersynchronous bursting activity they could be employed to compare the amount of high voltage, slow activity in the control and post-injection records.

The data from each experiment were handled by two somewhat similar methods.

The first method involved a direct calculation

of the percentage change in the post-injection period compared to the control and was accomplished in the following steps:

> (1) The height of the 30 dynograph recordings for channels 1, 2 and 3 of the analyzer were measured in both periods.

> (2) The measurements were totaled separately for each period.

> (3) The difference between the sums in (2) was taken, divided by the sum for the control period and multiplied by 100.

The results for this first method are listed in table 6 under the columns headed "area."

The second method involved a comparison of the percentage of electrical activity appearing in the first three channels of the analyzer to that occurring in all 8 channels during each period. The percentage change in the post-injection period was then calculated as in the first method. The procedure was an follows:

> (1) The heights of the dynograph records for all 8 channels of the analyzer were measured in both control and post-injection periods.

> (2) The measurements taken in (1) were totaled for each channel.

TABLE 6

Percentage of post-drug increase over the alert
control values for analyzer channels 1, 2, and 3.

DRUG	DOSE mM/kg	CONSCIOUS STATE	CAT CI-2 AREA	CAT CI-2 RATIO	CAT CI-4 AREA	CAT CI-4 RATIO	CAT CI-5 AREA	CAT CI-5 RATIO
TMA	.005	Drowsy	21.4	5.9	51.5	26.1	1.7	11.8
	.01	Alert	23.7	12.2	103.6	51.0	82.1	23.8
MDA	.005	Drowsy	35.4	10.0	45.7	8.2	18.4	0
	.01	Alert	7.1	14.9	70.4	34.0	45.2	7.6
MMDA	.02	Drowsy	54.8	23.8	-	-	73.6	14.7
	.03	Drowsy	-	-	216.7	64.2	-	-
	.04	Alert	20.2	14.7	28.6	6.8	90.1	26.5
DMA	.02	Drowsy	23.2	5.2	58.6	13.0	56.6	2.3
	.04	Alert	16.5	5.7	22.9	2.0	40.6	3.2
MESCA-LINE	.02	Drowsy	19.2	3.0	-	-	-	-
	.04	Drowsy	-	-	84.1	20.0	14.8	0.5
	.08	Alert	21.7	16.4	36.0	7.6	74.2	13.7

(3) A grand total for each period was computed from the results in (2).

(4) A subtotal for channels 1, 2 and 3 in each period was computed from the results in (2).

(5) The subtotals in (4) were divided by the grand totals in (3) and multiplied by 100.

The results for the second method are listed in table 6, under the columns headed "ratio."

A designation of either "alert" or "drowsy" will be found in table 6 opposite each dose of the test compounds. The term "alert" indicates that the animal was observed to remain in this state for the entire 30 minutes of the post-injection period and hence, for reasons previously discussed, this would be considered an "effective" dose of the compound. The term "drowsy," on the other hand, denotes an "ineffective" dose since the drug failed to maintain the animal in a constant state of alertness during this period.

In order to obtain quantitative comparisons of the potency of the various amphetamine derivatives TMA, the most potent compound in the series for producing hypersynchronous slow wave activity in the brain of the cat, was selected as the standard. The "effective" dose of TMA was found to be .01 mg/kg and the

responses it produced at this level were equated to
100%. The other test compounds were compared to TMA
according to the following formula:

$$\% \text{ TMA potency} = \left(\frac{\text{drug response}}{\text{effective dose}} \bigg/ \frac{\text{TMA response}}{.01 \text{ mM/kg dose}} \right) \text{ X } 100$$
$$\text{in mM/kg}$$

The results of these comparisons are listed in
table 7.

Of the two reference compounds which have been
used in these experiments only the data for mescaline
are presented. Amphetamine has not been included for
reasons previously discussed.

The reason why the data were compared by two
methods requires clarification and might best be ex-
plained by an examination of figure 5. It will be
noted, upon comparing the histograms for any one ex-
periment in this figure that two types of change occur
in their patterns; one is an alteration in the shape
of the histogram and the other is an increase or de-
crease in its over-all area. This would seem to in-
dicate that not only do fluctuations occur in the
relatively narrow range of frequencies integrated by
any one channel but also that fluctuations occur
simultaneously over a number of channels as the animal
changes from one state to another.

The histograms opposite the "numerical" scales

TABLE 7

Percentage of TMA response for post-injection increases over
alert control valves for analyzer channels 1, 2 and 3.

DRUG	EFFECTIVE DOSE (mM/kg)	CAT CI-2		CAT CI-4		CAT CI-5	
		AREA	RATIO	AREA	RATIO	AREA	RATIO
TMA	.01	100	100	100	100	100	100
MDA	.01	30	120	70	67	55	32
MMDA	.04	24	30	7	3	27	28
DMA	.04	17	12	6	1	12	3
MESCALINE	.08	11	17	4	2	11	7

in figure 5 depict changes in both area and shape; the
calculations made by the first method, and listed
under the "area" columns in figure 6, were taken from
data similar to that illustrated in this type of graph.
Alterations in area have been eliminated from the
histograms appearing opposite the "percent" scales in
figure 5; this was accomplished by expressing the
activity in each channel as a percentage of the total
activity in all channels. Changes of frequency within
the individual channels of the analyzer are emphasized
by this type of presentation and data similar to this
were used to make the calculations in the second.
method which appear under the "ratio" columns of fig-
ure 6.

No preference can be given to either type of
data treatment since nothing at all is known concern-
ing the complex interrelationships of frequency and
energy level changes which summate to produce the pat-
terns of electrical activity which we can observe in
the brain. The data were tabulated by these two
methods in the hope that at least one of them would
yield consistent, quantitative results in all three
experimental animals.

4. Conclusions

It can be seen by inspection of table 7 that the
results obtained with Cat CI-4 did not agree closely with

those from Cats CI-2 and CI-5. This fact eliminates the
possibility of making relatively sharp quantitative com-
parisons for potency among the various test compounds.
The data, however, will apparently support the following
conclusions:

    (1) TMA and MDA are the most potent compounds in
        this particular series of amphetamine deriva-
        tives for producing hypersynchronous, slow
        wave bursting activity in the brain of the
        conscious cat. TMA appears to be more active,
        in this regard than MDA.

    (2) DMA is clearly the least active compound.

    (3) MMDA has a potency somewhere between that of
        TMA and MDA on one hand and DMA on the other.

    (4) The reference compound mescaline has a potency
        similar to that for DMA.

    (5) The reference compound amphetamine does not
        produce hypersynchronous bursting at the doses
        tested.

While these experiments did not yield the consist-
ent quantitative results which were desired, they did suc-
ceed in ranking the test compounds in a fashion somewhat
analogous to what is known concerning their relative po-
tency as hallucinogenic agents in man. TMA, for instance,
is known to be more active than mescaline in man and in
the cat it was also clearly more potent that the latter

drug. The magnitude of differences varied between the two conditions since in man TMA is approximately 5 times more potent than mescaline while in the cat it is at least 10 times more active. MDA, in one experiment, was reported to produce hallucinations in man at a dose which would be comparable to that necessary for TMA and the two compounds also have similar degrees of activity for eliciting hyper-synchronous bursting in the brain of the cat. DMA produced hallucinatory phenomena in one schizophrenic patient at a dosage level which would also be effective for mescaline and in the cat these two compounds showed a similar potency for producing bursting activity. While no information is available concerning the hallucinatory activity of MMDA, it has been judged to be less than one-half as active as TMA for producing threshold central effects in man and in the cat MMDA is clearly the less active compound of the two. Amphetamine is not considered to be a hallucinogenic agent in man and it did not produce hyper-synchronous bursting activity in the brain of the cat.

The similarities between the two situations indicate that some empirical relationship may exist between the ability of these compounds to produce hypersynchronous bursting activity in the brain of the cat and their ability to produce hallucinatory phenomena in man although more data concerning the effects of these drugs in both species would be necessary to define this relationship

clearly.

D. COMPARING THE RATIOS OF PRE-DRUG TO POST-INJECTION EFFECTS DURING A CLASSICAL CONDITIONING PROCEDURE

1. Introduction

An adversive conditioning procedure was employed in this phase of the investigation into the effects of the amphetamine derivatives on ongoing brain electrical activity in the cat. It was undertaken with two goals in mind. The first of these was to produce, if possible, a relatively uniform state of alertness in the experimental animals which should have resulted in a more stable EEG pattern and hence in a less variable analyzer control record against which drug effects could be compared. The second objective was to discover what effects, if any, the test compounds might have on the electrical manifestations of a classically conditioned alerting response.

The attempts to produce a uniform degree of alertness in cats was only partially successful. The decision was made to use a blast of air as an unconditional alerting stimulus rather than the more commonly employed electric shock. This decision stemmed from a desire to eliminate blocking of the electronic recording instruments which often occurs when electrical current is caused to flow in the experimental animal. The initial concept was to deliver a single sharp puff of air to the back of the

animal's neck via a Tygon tube affixed to a modified harness. One cat (CI-6) was tested under these conditions. It rapidly became apparent that this procedure would not serve to keep the animal alert since the cat very quickly learned that the best way to avoid the air blast was to lay down and pull its head to one side. This position was similar to that often adopted while sleeping and the animal often proceeded to do just that.

A further modification of the air delivery system was tried; rather than one tube fastened near the midline, a pair of tubes were placed more laterally on either side of the harness. In addition to this bilateral placement the orifice of each tube was considerably reduced by cementing in a section of rigid, small diameter, plastic tubing. Cat CI-6 was again tested using this modification but in spite of enough air velocity literally to blow fur off the animal, it again experimented until it found a relatively "safe," curled position and proceeded once more to go to sleep.

A third modification of the system was undertaken when the notion of "flapper" tubes was conceived. The "flapper" tubes were 14 cm. lengths of soft, flexible, rubber tubing having an internal diameter of 1.5 mm. and a wall thickness of 1.0 mm. They were cemented firmly into the ends of two lengths of Tygon tubing which branched off from a glass "Y" tube, the other end of which was connect-

ed through a long length of Tygon tubing to a source supplying compressed air at 90 lbs./in.^{2}. This apparatus was fastened to the cat by slipping the short lengths of Tygon tubing, with their attached "flapper" tubes, through the loops of two leather thongs laced into both dorsal straps on each side of an ordinary small animal harness. The tubes were inserted through these loops and the thongs were pulled firmly through their lacings so as to secure the Tygon tubes to the harness. This positioned the "flapper" tubes so that they hung limply down each side of the cat's neck. When air was delivered to this system it caused the "flapper" tubes to flay violently about with the generation of considerable noise. Since the cats could not escape the violent, noisy action of the tubes regardless of what position they adopted the system, at least in the initial phases of the experiments, was successful in producing the desired behavioral and electrical alerting.

2. Procedure

Three cats with chronically implanted electrodes were used in the study; two males and one female. These animals were classically conditioned in an adversive situation which involved pairing a 3000 cycle per second tone (CS) with a "flapper" tube air blast (US). The CS was presented to the animal for 5 seconds prior to the US and then both remained on for one second prior to terminating

together. The intertrial interval was fixed at one minute. During testing procedures the animals were housed in a Lehigh Valley Company, sound attenuated, recording chamber which was equipped with a one-way mirror.

For a period of several days the cats were given a series of CS-US pairing. All very quickly established the conditioned response (CR), which was defined as EEG "alerting" or "desynchronization" upon presentation of the CS alone. The CR was accompanied by certain behavioral manifestations which will be more fully described later.

Once the CR was well established, the animals were put on a fixed ratio-variable interval conditioning schedule in which the CS was randomly paired with the US for one-third of the trials.

The experiments to determine the effects of the amphetamine derivatives, and the two reference compounds, mescaline and amphetamine, on the frequency distribution and conditioned alerting response in the EEG of the cat were carried out in the following manner:

The animals were placed in the recording chamber after being "hooked-up" to the air delivery and electrical recording systems and were allowed at least 30 minutes to become settled in this environment. The recording instruments were started and a series of 24 CS presentations were given. Eight of these were paired with the US in a random order. The cat was then injected intraperitoneally

with 2 ml. of a solution containing either NaCl at .04 mM/kg or one of the test compounds in doses from .005 to .08 mM/kg. A period of observation was begun during which any drug induced changes were noted and recorded. At that point in time, following drug administration, when the analyzer record showed obvious and relatively stable shifts, the series of 24 CS presentations, with random US pairings, was again repeated. In the event that no definite analyzer or behavioral changes were observable, the second set of trials was started between 30 and 60 minutes post-injection. The analyzer record ran continuously during the entire course of the experiment but the EEG was taken continuously only during the two series of 24 trials. The three cats were tested on the same days with 24 hours elapsing between each session. The sequence of their appearance on any given day was systematically rotated and the saline and various drug doses were given in random order. A total of 17 experiments were conducted on each animal.

3. **Electrode Placements**

Cat CI-6

Male, short haired, black and white weighing 3.4 kg.
The electrodes are calculated to be in the following locations (Jasper and Ajmone-Marsan):

AREA	STEREOTAXIC COORDINATES		
Left dorsal hippocampus	F+ 4.5	L5	H+7.5
Right midbrain reticular formation	F+ 3.0	L3	H-1.0
Left diagonal band of broca	F+ 16.0	L3	H-4.5
Right caudate nucleus	F+ 17.0	L5	H+5.0

Cortical electrodes were placed bilaterally in the frontal, parietal and occipital areas.

Cat CI-7

Male, short haired, orange tiger weighing 3.4 kg.

AREA	STEREOTAXIC COORDINATES		
Left dorsal hippocampus	F+ 4.5	L5	H+7.5
Right midbrain reticular formation	F+ 3.0	L3	H-1.0
Left diagonal band of broca	F+ 16.0	L3	H-4.5
Right caudate nucleus	F+ 16.0	L5	H+5.0

Cortical electrodes were placed bilaterally in the frontal, parietal and occipital areas.

Cat CI-8

Female, long haired, black and tan weighing 3.2 kg.

AREA	STEREOTAXIC COORDINATES		
Left dorsal hippocampus	F+ 4.5	L5.0	H+7.5
Right midbrain reticular formation	F+ 3.0	L3.0	H-1.0
Right n. medialis dorsalis of the thalamus	F+ 7.5	L2.5	H+3.5
Left lateral geniculate body	F+ 7.5	L10.0	H+2.5

Cortical electrodes were placed bilaterally in the frontal, parietal and occipital areas.

4. Results

    a) The Conditioned Response vs. The Test Compounds

Cat CI-6 did not develop a satisfactory conditioned alerting response. One reason for this might have been the previous experience which this animal had with "ineffective" air delivery systems (see Introduction, this chapter). The tone would usually produce EEG alerting for several trials following a CS-US pairing but if the random order dictated the occurrence of more than 2 or 3 consecutive, unreinforced CS presentations the CR would often fail to appear.

Cat CI-7 developed a conditioned alerting response quickly. As the experiments continued, however, a degree of adaptation occurred. In the later sessions, the CR would occasionally fail to appear if a relatively long series of unreinforced CS presentations were given.

Cat CI-8 also quickly established a conditioned alerting response. Unlike the other two animals no adaptation occurred and the CR remained constant during the entire series of experiments.

The effects of TMA on the conditioned alerting response are depicted in figure 6 and can be taken as typical for the activity of the other three amphetamine derivatives as well as for mescaline. It will be

noted that under control conditions, the EEG record demonstrates low voltage, fast activity extending well beyond the CS interval. Following administration of TMA, however, the typical hypersynchronous bursting response produced by this compound interrupts the initial desynchronization produced by the CS. In a number of experiments the bursts became even more pronounced following termination of the CS. This can be seen, to some degree, in the record for CI-6 in figure 6.

In general, the disruption of the conditioned alerting response was, to some extent, dose related. This was true in the sense that administration of larger amounts of a given agent would often produce this effect in a greater percentage of trials. Complete failure to show any alerting of the EEG was comparatively rare, although it was seen on occasions.

Since amphetamine did not result in hypersynchronous bursting but, on the contrary, produced faster frequency, low amplitude "alert" patterns, no such phenomena as depicted in figure 6 was observed following its administration.

The behavioral manifestations of the conditioned response, as opposed to the electrical desynchronization of the brain, showed considerable variation between individuals as well as variations in the one

Control   CI-6   Post Injection

Control   CI-7   Post Injection

Control   CI-8   Post Injection
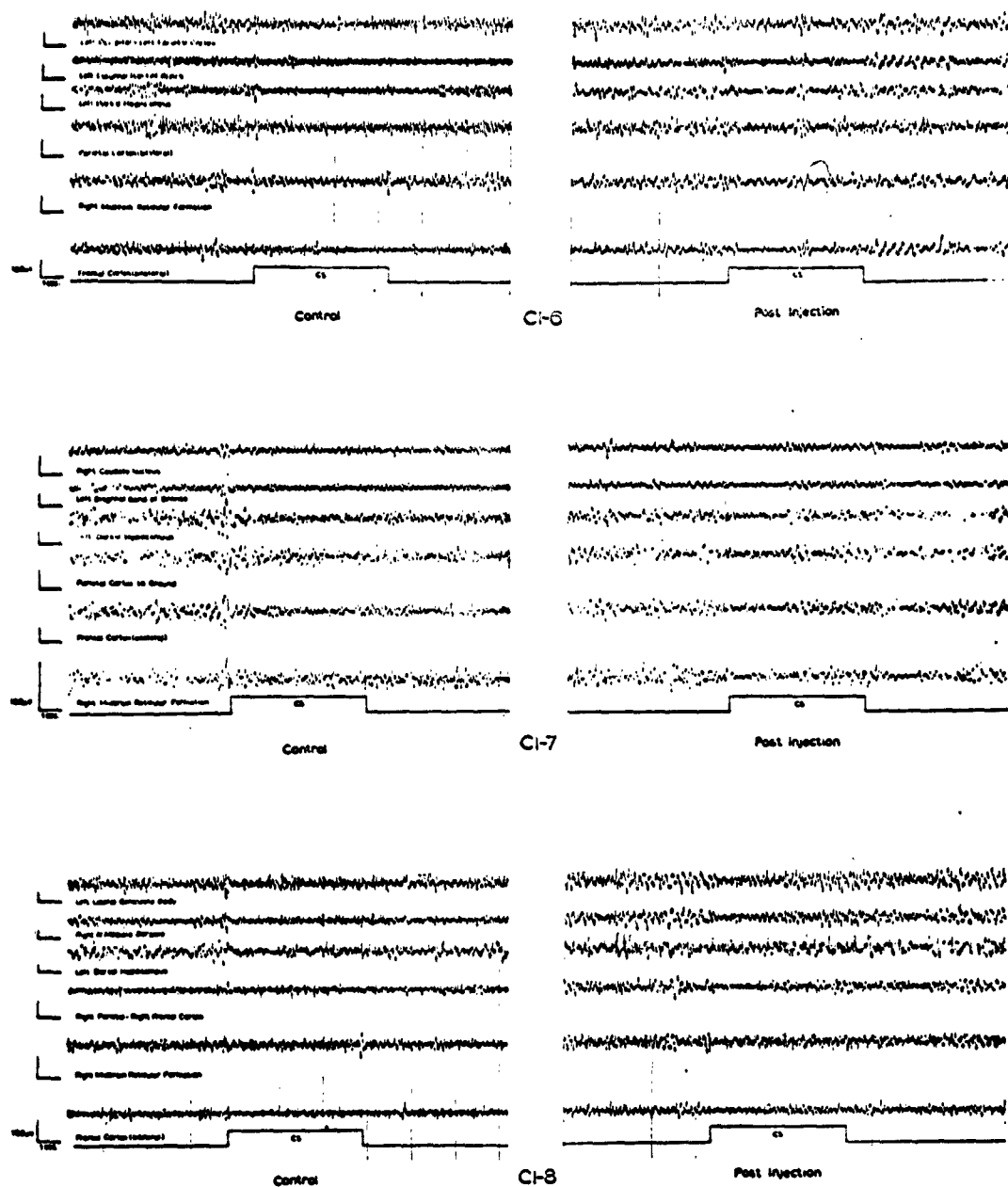
These records illustrate the conditioned alerting response in 3 unanesthetized and unrestrained cats before and after the injection of .02 m. mol. / Kg. TMA.

The CS interval is indicated by the elevated segment of the lower, horizontal line.

Figure 6

animal during different experiments. For instance, during the control periods Cat CI-6 would usually display no observable response to the onset of the CS and Cat CI-7 would often respond with only slight movements of the head or ears, although it would occasionally orient its attention toward the speaker. Cat CI-8, on the other hand, quickly adopted a complex reaction pattern which became almost stereotyped during the course of the experiments; this animal would characteristically crouch at the onset of the tone and pull its head around to one side keeping its chin as low to the cage floor as possible.

The reaction of the cats to the US (air blasts delivered through the flapper tubes) during the control periods also varied to some extent. The two male animals, CI-6 and CI-7, would often bear the ordeal stoically and merely close their eyes and flatten their ears, while the female, CI-8, would usually move her body back from her crouched position and then "meow" and rise briefly to her feet.

The behavioral patterns exhibited to the CS and US following injection of the test compounds was likewise complex and variable. About the only conclusions which can be drawn concerning these effects is to say that in general the drugs tended to enhance behavioral responses to the CS and diminish those to the US.

This phenomenon is best illustrated by describing a typical reaction of Cat CI-8 to higher doses of the more potent drugs in this series. This animal, as previously mentioned, had developed a more or less stereotyped, behavioral CR and showed quite a marked reaction to the US. Following administration of the drugs presentation of the CS would usually elicit a more vigorous "crouching" behavior which was accompanied by what appeared to be repeated "hissing" reactions. The cat would often begin retreating before onset of US but when the air hit the flapper tubes she would paradoxically stop and look about in a startled fashion as the tubes flayed about her head; she often did not appear to be as disturbed by their action as during the control sessions. This general phenomenon was also reflected in Cats CI-6 and CI-7 but to a less dramatic degree.

Amphetamine usually produced considerable motor activity in the experimental animals although it more often took the form of rapid and continual head movements from a sitting or crouched position rather than general locomotion. Presentation of the US and CS usually did not alter this picture. The other compounds in the series usually resulted in relative immobility during the intratrial intervals with increased activity after presentation of the CS. There

were exceptions to this, especially with Cat CI-6.
This animal often became quite excited following drug
injection, although this behavior apparently did not
occur with any specific compounds nor did it appear to
be dose related. For instance, following administra-
tion of .04 mM/kg mescaline the animal became so
hyperactive that electrical recording became very
difficult, although such a response was not seen with
either .02 or .08 mM/kg of the same compound.

b) Quantitative Estimates of Relative Drug Potency

As previously mentioned, one of the original
objectives of the classical conditioning procedure
employed in this investigation was to produce a con-
tinuous state of alertness in the animal. This would
have resulted in an EEG pattern largely devoid of low
frequencies which would have provided a relatively
stable base line for comparing drug induced hypersyn-
chronous bursting activity in the various cats. Since
this goal was only partially realized the pre-drug EEG
contained variable amounts of low frequency waves
which to some extent made the comparison of the pre-
and post-injection frequency distributions more diffi-
cult. An analysis of variance was performed on the
data to establish the statistical significance of
certain drug induced changes and to separate the vari-
ation due to different experiments, different drugs,

different doses of the same drug and different channels of the frequency analyzer. Each experiment served as its own control and the analysis was performed on the ratio of the amplitude in each channel after the drug to the amplitude in the control period. These results are presented in tables 8, 9 and 10. Significant differences were found between channels, drugs and doses of each drug in both cats CI-7 and CI-8. However, no significant differences were found for these parameters in cat CI-6. This animal, as was previously discussed, did not develop a satisfactory CR and for this reason remained drowsy during the control period a greater percentage of the time. This drowsiness introduced high voltage, slow wave activity into the control record which had a tendency to mask the drug induced hypersynchronous bursting activity occurring in the same frequency range.

A number of interactions proved to be highly significant. The linear regression of both drugs on channel number and doses on channel number (indicating a linear regression on the logarithm of the frequency) was significant in all cats. The linear regression coefficients (a) were calculated and are presented in table 11. Since the linear regression on channel numbers was significant the potency of each drug could be estimated from the slope of "a" when plotted against

TABLE 8

Analysis of Variance

Cat CI-6

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Between Channels:					
Linear Regression	2.30067	1	2.30067	172.20	<.001
Quadratic Regression	1.93607	1	1.93607	144.90	<.001
Residual	0.06681	5	0.01336		
Between Drugs	1.70068	6	0.28344	1.51	>.20
Between Doses, except Saline	1.06222	9	0.11802	-	-
Between Saline Replicates	0.37515	1	0.37515		
INTERACTIONS with CHANNELS					
(A) Drugs:					
(1) Linear Regression	1.80221	6	0.30036	10.90	<.001
(2) Quadratic Regression	1.07939	6	0.17989	6.53	<.001
(3) Residual	0.82648	30	0.02755		
(B) Doses except Saline:					
(1) Linear Regression	0.62777	9	0.06975	5.75	<.001
(2) Quadratic Regression	1.00283	9	0.11142	9.19	<.001
(3) Residual	0.54527	45	0.01212		
(C) Saline Replicates	0.15560	7	0.02223	-	-
TOTALS	13.48115	135			

TABLE 9

Analysis of Variance

Cat CI-7

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Between Channels:					
Linear Regression	2.69917	1	2.69917	17.40	<.01
Quadratic Regression	0.04566	1	0.04566	00.29	-
Residual	0.77565	5	0.15513		
Between Drugs	4.50011	6	0.75001	117.18	<.001
Between Doses, except Saline	0.81318	9	0.09035	14.12	<.001
Between Saline Replicates	0.00640	1	0.00640		
INTERACTIONS with CHANNELS					
(A) Drugs:					
(1) Linear Regression	2.36832	6	0.39472	20.84	<.001
(2) Quadratic Regression	0.45158	6	0.07526	3.97	<.01
(3) Residual	0.56829	30	0.01894		
(B) Doses, except Saline:					
(1) Linear Regression	0.90453	9	0.10050	14.24	<.001
(2) Quadratic Regression	0.09010	9	0.01001	1.42	-
(3) Residual	0.31771	45	0.00706		
(C) Saline Replicates	0.03180	7	0.00454	-	-
TOTALS	13.57251	135			

TABLE 10

Analysis of Variance

Cat CI-8

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Between Channels:					
Linear Regression	.41917	1	.41917	15.61	<.05
Quadratic Regression	.04992	1	.04992	1.86	-
Residual	.13427	5	.02685		
Between Drugs	.75056	6	.12509	20.84	<.01
Between Doses, except Saline	.37864	9	.04207	7.00	<.05
Between Saline Replicates	.00601	1	.00601		
INTERACTIONS with CHANNELS					
(A) Drugs:					
(1) Linear Regression	.47828	6	.07971	17.27	<.001
(2) Quadratic Regression	.06706	6	.01118	2.42	<.05
(3) Residual	.13846	30	.00461		
(B) Doses except Saline:					
(1) Linear Regression	.32648	9	.03628	14.71	<.001
(2) Quadratic Regression	.07096	9	.00788	3.19	<.01
(3) Residual	.11101	45	.00247		
(C) Saline Replicates	.01594	7	.00228		
TOTALS	2.94676	135			

TABLE 11

(a)

DRUG	DOSE (mg/kg)	Linear Regression Coefficients				Quadratic Regression Coefficients			
		CI-6	CI-7	CI-8	MEAN	CI-6	CI-7	CI-8	MEAN
SALINE	No. 1	-.038	-.009	+.009	-.013	+.021	+.019	+.001	+.014
	No. 2	-.004	0	-.015	-.006	0	+.006	-.002	+.001
TMA	01	-.138	-.096	-.032	-.089	-.065	-.013	-.005	-.028
	02	-.168	-.116	-.048	-.111	-.101	-.024	+.015	-.037
MDA	01	-.107	-.054	-.053	-.071	-.070	+.004	+.007	-.020
	02	-.074	-.075	-.048	-.066	-.098	+.027	-.019	-.030
MMDA	01	-.071	-.040	-.024	-.045	-.006	+.018	+.006	+.006
	02	-.032	-.114	+.004	-.047	-.031	+.007	+.025	0
	04	-.103	-.236	-.101	-.147	-.007	+.004	-.011	-.005
DMA	02	-.031	-.019	-.007	-.019	-.016	+.015	+.018	+.006
	04	-.027	-.035	-.061	-.041	-.048	+.010	+.010	-.009
	08	-.020	-.027	-.032	-.030	-.116	+.020	+.034	-.021
MESCALINE	02	-.093	-.085	-.038	-.072	+.040	+.050	+.014	+.035
	04	+.009	-.110	-.022	-.041	-.056	+.030	+.026	0
	08	-.129	-.120	-.020	-.090	-.093	+.049	+.020	-.008
AMPHETAMINE	005	+.053	+.026	+.032	+.037	-.056	-.017	+.009	-.031
	04	+.006	+.064	+.043	+.038	-.180	-.070	-.007	-.086
Standard Error		±.021	±.018	±.010		±.021	±.018	±.010	

dose. This slope was estimated for each drug as a linear regression coefficient and the results are summarized in table 12.

In addition to linear regression, quadratic regression on the channel numbers for both drugs and doses was found to be significant for cats CI-6 and CI-8. In cat CI-7 significant quadratic regression was found for drugs but not for doses. No specific explanation can be offered for this divergent result in CI-7 although it probably reflects random variation. On pooling the components for quadratic regression for all three cats this factor was found to be highly significant ($F = 5.85$, $n_1 = 27$, $n_2 = 135$, $P = < .001$).

The hypersynchronous bursting activity produced by the test compounds had a frequency range which corresponded to the band pass for the first three channels of the analyser and these channels were previously used to compare the activity of the test compounds (section C of this chapter). In order to have a corresponding analysis from the current experiments the relative drug activity at channel 2 was calculated by determining the regression of the mean slope on the dose of the drug at channel 2 from the linear and quadratic regression coefficients pooled for all three cats. These results appear in table 13.

TABLE 12

Slope Comparison by Drug Dose

Drug	Cat CI-6 Relative Activity	Percent TMA	Cat CI-7 Relative Activity	Percent TMA	Cat CI-8 Relative Activity	Percent TMA	Mean Percent TMA
TMA	9.5	100	6.6	100	2.6	100	100
MDA	5.1	54	4.1	62	3.0	115	77
MMDA	2.7	26	5.8	88	2.0	77	64
DMA	0.5	5	0.5	8	0.6	23	12
Mescaline	1.4	15	1.9	29	0.4	15	20

TABLE 13

Mean Slope at Channel 2

DRUG	DOSE	MEAN SLOPE	REGRESSION OF MEAN SLOPE ON DOSE OF DRUG	PERCENT TMA
TMA	.01	-.229	16.4	100
	.02	-.296		
MDA	.01	-.171	12.1	74
	.02	-.216		
MMDA	.01	-.015	3.8	23
	.02	-.047		
	.04	-.172		
DMA	.02	+.011	1.1	10
	.04	-.086		
	.08	-.135		
MESCALINE	.02	+.103	1.2	7
	.04	-.041		
	.08	-.130		
SALINE	-	+.057	-	-
	-	-.001	-	-

5. Conclusions

a) The Conditioned Response

The particular air-blast technique used in these experiments was not fully adequate in sustaining the conditioned alerting response. In order to realise the original goal of keeping the cat in a constant state of alertness a more adversive stimulus than that employed would evidently be necessary.

The CR usually interrupted drug-induced hypersynchronous bursting in the electrical activity of the brain of the cat but this effect was often only momentary and bursting would again appear prior to termination of the CS. This effect had a degree of inverse correlation to dosage level since less "CR breakthrough" would generally occur following injection of greater amounts of the test compounds.

Behavioral manifestations of the CR were apparently increased by the test compounds and the impression was gained that the CS became more adversive to the cat; on the other hand, adversive reactions to the US seemed to be decreased by these drugs.

b) Quantitative Comparison of Drug Potency

An inspection of table 12 will reveal that appreciable variation occurred in the relative activity of the amphetamine derivatives between the three cats as calculated by the slopes of the linear regression.

coefficients on channel numbers. This fact makes a
close quantitative comparison unrealistic but the data
will support the following conclusions:

(1) TMA and MDA are the most potent compounds with
TMA probably being the more active of the two.

(2) DMA is the least potent amphetamine derivative
in this series.

(3) MMDA has an activity which falls somewhere
between these extremes.

(4) The reference compound mescaline has a potency
which is more similar to DMA than the other
amphetamine derivatives.

When considering the regression of the mean
slope on the dose of the drug at channel 2 (table 13)
similar relationships to those discussed above are re-
vealed. It will be noted that mean slopes at channel
2 appear to be dose related. This appears in contrast
to the linear and quadratic regression coefficients in
table 11 which were calculated from all 8 channels of
the analyzer and show less consistent relationship
with dose. The current experiments, in which the ani-
mals were subjected to an aversive conditioning proce-
dure in an attempt to maintain a consistent pre-drug
state of alertness, ranked the compounds in a fashion
analogous to that for the first 3 cats where the alert
state was maintained under direct observation and

manipulation by the experimenter.

Amphetamine, while producing a number of significant changes in the frequency distribution of the cat EEG, resulted in patterns essentially opposite to those elicited by the other test compounds since it generally produced a decrease in the lower frequencies and an increase in the higher ones (note the positive linear regression coefficients for amphetamine in table 11).

None of the 6 saline control experiments produced any significant changes in the frequency distribution.

The relationships between the frequency changes in the brain of the cat, as observed in these experiments, and the hallucinogenic potency of the test compounds in man were essentially similar to those previously discussed in part D, section 4 of this chapter.

E. COMMON DRUG EFFECTS IN UNANESTHETIZED CATS EMPLOYED IN THE INVESTIGATION OF BRAIN ELECTRICAL ACTIVITY

1. Hypersynchronous Bursting Activity

Chronically indwelling bipolar electrodes were calculated to be situated in eight subcortical sites which included the dorsal hippocampus, midbrain reticular formation, amygdaloid area, septal region, nucleus medialis dorsalis of the thalamus, diagonal band of Broca, head of

the caudate nucleus and the lateral geniculate body.

Cortical electrodes were placed in the frontal, parietal and occipital areas of the skull.

None of these placements was checked histologically.

Following injection of sufficient doses of mescaline and the four phenyl substituted amphetamine derivatives hypersynchronous bursting activity was seen, to a varying degree, in all of these brain loci. In general, the subcortical electrodes exhibited this phenomenon more clearly and with larger amplitude than did the cortical leads. Of the eight subcortical sites examined only the amygdaloid area and head of the caudate nucleus were relatively deficient in this regard.

The septal region and reticular formation placements, as a general rule, showed particularly clear hypersynchronous bursting. The dorsal hippocampal electrodes, while occasionally appearing less sensitive than other areas, frequently exhibited distinct bursting following drug injections. The nucleus dorsalis medialis of the thalamus and the diagonal band of Broca also showed clear bursting activity on most occasions.

There were, of course, many exceptions to the general impressions given above and variations were observed in the responses of a given animal to the different drugs as well as between cats to the same compound. Because of the wide distribution of drug-induced hypersyn-

chronous bursting activity in the brain, a histological verification of electrode placement was not undertaken. For this reason no precise information is available concerning the possible relationship of anatomical location to observed variations in drug activity.

There was apparently a fairly consistent difference in the frequency of the hypersynchronous activity produced by mescaline and that produced by the four amphetamine derivatives. Mescaline injected in sufficient amount was usually followed by 12 to 14 cycle per second high amplitude bursting in the form of sharp waves. The amphetamine derivatives, on the other hand, resulted in trains of slower, high amplitude activity between 4 and 7 cycles per second which were more sinusoidal in shape. Figure 3 clearly demonstrates this effect in one animal.

The ability of mescaline and the four amphetamine derivatives to produce high-amplitude, low frequency hypersynchronous bursting activity in the brains of conscious and unrestrained cats is a very real phenomenon. These experiments have shown this effect to be dose related for any given drug, and in addition have shown that the various compounds in the series vary in their potency for producing it.

Difficulties in obtaining quantitative information on comparative activity stemmed from at least two known sources; one instrumental and the other from an inherent

similarity of certain intrinsic brain rhythms to drug
induced bursting activity.

Since the basic unit of measure in these experi-
ments was the histogram produced by the write-out circuit
an eight channel frequency analyzer, the results are de-
pendent upon the reliability of this instrument. Once the
"trouble-shooting" period had passed it is felt that vari-
ations from this source were minimal. However, one con-
stant source of error was present throughout all of the
tests. Artifacts introduced into the record through move-
ments of the animal were never fully controlled. An
attempt was made to do so and although it was partially
successful more effort should have been expended in this
regard. This is particularly true in view of the fact
that both drug induced hypersynchronous bursting and move-
ment artifact appeared as slow wave, high amplitude activ-
ity to the frequency analyzer. Fortunately, all of the
test compounds, except amphetamine, often resulted in
marked reduction of spontaneous movement and thus error
from this source was probably of much less significance
than it might have been.

Another instrumental difficulty arose from the fact
that the analyzer was constructed with the frequency re-
sponse of its channels fixed instead of variable; had it
been possible to "tune-in" on the frequency of the drug
induced bursting sharper results might have been attained.

Such a modification is now being contemplated.

Another difficulty encountered in these experiments
has previously been discussed (section C, part 3 of this
chapter). The frequency of the drug-induced brain elec-
trical activity corresponded to normal brain rhythms oc-
curring when the animal was drowsy or in certain stages of
sleep, although a major difference in the regularity of
the two patterns made it simple to distinguish them when
comparatively high doses of the drugs were used. Under
circumstances of high dosage, the variability of the min-
ute to minute analyzer patterns was much less following
the drug administration than during periods of drowsiness,
and in addition, the animal appeared obviously "alert."
When working with threshold doses of the test compounds
the situation became more difficult to interpret and slow
wave contribution from drug activity became easily con-
founded with similar, naturally occurring frequencies. As
previously described, various environmental manipulations
were resorted to in an attempt to overcome this difficulty
and keep the animal continually alert. Classical condi-
tioning with a truly adversive unconditional stimulus
could possibly have resolved this problem.

The placement of electrodes in all probability in-
fluenced the results to some extent even though the hyper-
synchronous bursts appeared widely distributed in the
brain. No attempt was made during these investigations to

elucidate this problem of varying responses from electrode placement although the importance of doing so is recognised.

2. Observed Physiological and Behavioral Changes

In the relatively large number of individual experiments conducted during the course of this investigation, the variability of response mentioned above in discussing hypersynchronous bursting also applied to drug induced behavioral and physiological changes. However, in spite of these differences, certain common characteristics in drug effect seem to rise above the background "noise-level" to the point where they can be discussed in terms of "general phenomena."

The ability of these compounds to produce what appears to be a behaviorally "alert" state and the various "staring" and "hissing" reactions which accompany it have been previously discussed. Concomitant with this "alert" state the cats were often paradoxically immobile following the injection of mescaline and the amphetamine derivatives. In a number of experiments these animals would maintain one attitude for several hours. As mentioned previously, the most common position was prone with the limbs extended and the head held rigidly upright.

In keeping with the general nature of their molecular structure a number of peripheral effects of these drugs appeared sympathomimetic in nature. Effects of this

type included mydriasis, piloerection and the flow of thick, viscous saliva. In addition, hyperpnea, urination, defecation and vomiting were often seen.

TMA, MDA and MMDA generally appeared more potent in producing sympathomimetic reaction that either mescaline or DMA. Mydriasis was the most consistent effect observed with the first three compounds while mescaline and DMA elicited this response only in the higher dosage ranges. Although a number of these peripheral effects appeared sympathomimetic in nature it will be recalled that in the studies on the transcallosal evoked potential only MDA proved to be a pressor agent in cats. Mescaline and the other three amphetamine derivatives caused marked falls in blood pressure.

Mescaline was the most potent emetic agent in this series of compounds and also resulted in a higher incidence of defecation.

Marked hyperpnea was a common observation, particularly following the injection of larger doses of TMA, MDA and MMDA; in some instances the respiratory rates rose well above 200 per minute.

F. GENERAL DISCUSSION AND REVIEW OF THE LITERATURE CONCERNING THE EFFECTS OF MESCALINE ON SPONTANEOUS BRAIN ELECTRICAL ACTIVITY

1. Introduction

The hallucinogenic agent lysergic acid diethylamide

(LSD) produces effects very similar to mescaline but has a
potency which exceeds the latter compound by at least 3000
to 4000 times. Probably because of this significantly
greater potency, LSD has been investigated more extensive-
ly than mescaline in recent times and a large amount of
literature exists concerning its effects. There is some
evidence that the two compounds may act on similar central
mechanisms. Szara (1957) pointed out that "equivalent"
doses of mescaline and LSD in man produce central phenom-
ena which are very similar and seem to differ mainly in
their time course (mescaline is slower in onset and has a
longer duration). A more recent article by Hollister and
Hartman (1962) confirmed these observations when they re-
ported that under carefully controlled conditions of dos-
age and environment mescaline, LSD, and another hallucino-
genic agent psilocybin, elicited very similar subjective
and peripheral effects in man. They concluded that these
three compounds "may have common mechanisms of action
despite their chemical differences." Cross-tolerance
phenomena have been demonstrated in man between LSD and
mescaline (Balestrieri, 1957; Balestrieri and Fontanari,
1959) and similar cross-tolerance has been seen in rats
(Freedman, Aghajanian and Ornitz, 1958). Rinaldi and
Himwich (1955c) investigating drug induced EEG changes in
the brain of the rabbit stated that LSD and mescaline pro-
duced "practically identical effects."

In spite of the above evidence for a common mode of action with mescaline, the vast literature on LSD will not be separately covered in this thesis; the compound will only be mentioned in conjunction with those publications in which both LSD and mescaline are investigated. The reasons for this are twofold; first, LSD was not employed in our studies and thus we have no experimental point of reference for discussing it; and second, we are not attempting to extrapolate the results of our investigation to include hallucinogenic compounds in general. Such an extension of the work could be considered a desirable goal but at present no attempt has been made to do so.

Of the six members of our test series only three compounds have been investigated for their electrographic effects by other laboratories and two of these three drugs (amphetamine and TMA) need not be discussed in any detail at this time. The EEG effects of amphetamine have been mentioned in numerous papers all of which report the production of an "alert" pattern in the brain consisting of low amplitude, high frequency electrical activity (Bradley and Elkes, 1957; Bradley and Key, 1958; White and Daigneault, 1958). These observations are consistent with our findings in the conscious cat. Only one report was found concerning the effects of TMA on brain electrical activity; Perets, Smythies and Gibson (1955) stated that 1.0-1.2 mg/kg did not significantly alter the EEG pattern in

man. These results are not consistent with our experiments in the conscious cat where TMA, in comparable doses, was observed to produce marked hypersynchronous bursting activity in the EEG.

Except for amphetamine, the only member of our test series of compounds which has been investigated to any extent for its ability to alter spontaneous brain electrical activity is mescaline. This drug will be discussed below in some detail.

2. The Effects of Mescaline on Spontaneous Brain Electrical Activity in Experimental Animals

The most characteristic change we observed in the electrical record from the brain of the unanesthetized cat, following administration of mescaline and the mescaline-like amphetamine derivatives, was a high amplitude, low frequency hypersynchronous bursting activity which we have mentioned many times in this chapter. A number of articles in the literature have reported similar phenomena following mescaline administration.

In an investigation which in many respects paralleled our own, Bradley and Elkes (1957) stated that mescaline in doses of 10 and 25 mg/kg resulted in no change in the EEG of conscious cats although 50 mg/kg produced a large amount of "rhythmic waves at 4-6 cycles per second" recorded from all areas of the cortex. In our experiments we routinely observed a similar phenomenon with doses of

.04 mM/kg (approximately 11 mg/kg) although this amount corresponds to a dose Bradley and Elkes found to be essentially inactive. The major difference between the two experiments was that they recorded exclusively from cortical sites while we also monitored deep structures. Hypersynchronous bursting activity, while usually discernible in cortical leads, was more pronounced in various subcortical sites (see figure 3); this may have drawn our attention to the fact that it was occurring in the cortex at the lower dosage ranges. Bradley and Elkes also reported that LSD produced a 4-7 cycle per second bursting activity.

Rinaldi and Himwich (1955c) working with conscious, but curarized, rabbits found that 10-20 mg/kg of mescaline injected intravenously caused an "alert" pattern with low voltage, fast activity in the cortex and also produced a 4-6 cycle per second rhythmic activity in thalamic sites. LSD at 10-15 micrograms/kg elicited similar effects and Frenquel (alpha-4-piperidyl benzhydrol hydrochloride) at 12-24 mg/kg blocked both the mescaline and LSD reaction within 2 to 10 minutes. The "alerting" produced by mescaline and LSD was mentioned by these authors in another publication (Himwich and Rinaldi, 1957) where they reported that Azacyclonal (alpha-4-piperidyl benzhydrol) was also capable of blocking the reaction. This "alerting" or "arousal" reaction was also reported in chronically im-

planted, conscious cats by Sailer and Stumpf (1957) following the injection of 30-40 mg/kg mescaline intravenously; no mention was made of slow wave, rhythmic activity and they likened the effect to that produced by 20-30 mg/kg of Pervitin (methamphetamine). In light of our experiments these results seem surprising.

Injecting 0.3-15 mg of mescaline directly into the third ventricle of the brain in conscious cats, Schwarz, Khalil, and Bickford (1956) observed 3-4 cycle per second sharp waves from all cortical electrodes with maximal activity in the temporal regions; these electrical phenomena occurred simultaneously with paroxysms of ear scratching and an odd yowling. In addition, long bursts of 10 cycle per second spikes were seen which alternated with low voltage, fast wave activity. From the descriptions these investigators gave of the electroencephalographic effects of intraventricular mescaline injections one gains the impression that what they were observing was, in a sense, an exaggerated case of what we found when administering the drug intraperitoneally. This is of interest with regard to reports by Block and Block (1952) and Block, Block and Patzig (1952) who found that at the height of the mescaline effect the amount present in brain tissue was very small while that in the liver was at a maximum. This fact, coupled with the long time of onset for central effect, led Block to speculate that the activity of mes-

caline was probably due to a secondary metabolic change.
Such a concept would seem hard to support in light of the
results obtained with direct, intraventricular injections
of the drug since in this situation the effects developed
rapidly, were very intense and resembled those seen fol-
lowing more conventional routes of administration.

According to a report by Speck (1958) the con-
scious, albino rat will also exhibit paroxysmal spiking
activity in its EEG following mescaline administration.
Relatively high doses (200-400 mg/kg) were necessary to
elicit this bursting response; lower doses produced the
"alert" or desynchronized EEG record which has been men-
tioned so frequently in conjunction with mescaline.

The final publication to be discussed stems from
Heath of Tulane University (Monroe and Heath, 1961). This
investigator and his co-workers have, for the past 10 or
12 years, been investigating various physiological aspects
of the schizophrenic process in man. Their work will be
covered in more detail in the following chapter where we
discuss possible future directions for our own research
efforts, but for the moment it is sufficient to say that
Heath's group believes that an abnormal paroxysmal spiking
pattern recorded from the subcortical septal area of the
human brain shows a high degree of correlation with the
incidence of psychotic behavior in a given subject. In
their paper, Monroe and Heath report that monkeys implant-

ed with electrodes in a comparable septal region, as well
as in various hippocampal sites, responded to mescaline
injection of from 9 to 80 mg/kg with "paroxysmal hypersyn-
chronous activity" which was confined to the aforemention-
ed septal and hippocampal structures. In addition, this
abnormal electrical activity was reported to occur simul-
taneously with drug induced behavioral changes (passivity,
catatonic-like posturing, etc.). LSD was found to produce
similar effects. A study of mescaline and LSD in human
subjects with chronically implanted electrodes had yielded
analogous results (to be discussed below) and these two in-
vestigations are cited by Heath as support for the theory
that abnormal, paroxysmal electrical activity in various
rhinencephalic structures of the human brain is closely
correlated with psychotic behavior. The inference by
Heath and co-workers that abnormal electrical patterns in
the brain of a monkey can be related to a psychotic pro-
cess in man naturally has considerable import for our ex-
periments in cats where we were able to demonstrate that
similar, dose related phenomena occurred following admin-
istration of mescaline. It would be most interesting to
discover if our group of test compounds would show approx-
imately the same potency relationships in the monkey
preparation as they did in the conscious cat.

In summary of the results obtained by various in-
vestigators, in a number of animal species, it could be

stated that mescaline administered in a sufficient dose to a conscious, intact animal would probably produce a type of hypersynchronous, slow wave bursting activity in various areas of its brain and at lower doses EEG "alerting" might occur. Our investigations in the cat confirmed the observation of hypersynchronous bursting but we failed to detect any marked alerting phenomena.

3. The Effects of Mescaline on Spontaneous Brain Electrical Activity in Man

Electrographic records taken from the scalp of subjects who have received mescaline generally do not exhibit remarkable alterations in pattern although a number of papers have mentioned various changes in the alpha rhythm (8-10 cycle per second activity). Chweitzer, Giblewicz and Liberson (1936) reported that alpha blocking occurred simultaneously with visual hallucinations while Rubin, Malamud and Hope (1942), on the other hand, stated that in schizophrenic subjects alpha frequencies were increased by mescaline. Endo (1952) found that mescaline initially increased and then depressed alpha rhythms and spike wave patterns reminiscent of convulsive discharges were also observed. Denber and Marlis (1956), in contrast to Endo, concluded that mescaline reduced or eliminated slow wave and spiking phenomena already present in the EEG while alpha blocking was also reported. Wikler (1954), working with post-addicts, reported that mescaline caused alpha

blocking in the same temporal sequence as visual hallu-
cinations in a number of his subjects, although some ex-
hibited no change and one showed an increase in alpha
activity.

While mescaline apparently produced variable
changes in the electrographic patterns from scalp elec-
trodes several reports in the literature indicate that the
activity induced by this drug in certain sub-cortical
structures is relatively consistent. As mentioned above,
R. G. Heath and co-workers have long been interested in
the physiology of schizophrenia and during the course of
their investigations developed techniques for chronically
implanting electrodes in various sub-cortical areas of the
human brain. In schizophrenic patients the electrical
activity recorded from certain rhinencephalic structures
was found to contain abnormalities in the form of slow
wave and spiking phenomena. Monroe, Heath, Mickle and
Llewellyn (1957) injected mescaline and LSD into a group
of these patients and found that the abnormal electrical
activity observed in the septal and hippocampal areas be-
came more intense and took on the form of high amplitude,
slow wave paroxysmal bursts; these patterns were said to
occur in conjunction with overt, psychotic behavior. A
year previous to this report Schwarz, Sem-Jacobsen and
Petersen (1956) published essentially similar results in
3 psychotic patients suffering from epilepsy plus 2

chronic schizophrenics. Both mescaline and LSD produced paroxysmal activity in the schizophrenics but the most striking result in the epileptics was a "pronounced quieting effect on the spike and sharp wave foci in depth recordings." This observation confirmed reports by Denber and Merlis (1956) who observed reduction of convulsive activity in scalp recordings.

The fact that mescaline produces a paroxysmal bursting activity in the brain of man which is apparently associated with disturbed or psychotic behavior is of considerable importance in attempting to extrapolate our results in the cat to relate to the human situation. We routinely noted a similar electrical disturbance in the cat which was definitely related to an abnormal, almost stereotyped behavioral pattern. These observations coupled with almost identical effects reported for the monkey (see above) lend support to the concept that paroxysmal bursting activity induced by certain drugs in various subcortical structures of the brains of sub-human species might successfully be correlated with the production of abnormal or psychotic behavioral responses in the human by the same chemical agents. Considerably more data than that which is currently available would be necessary to test the validity of this hypothesis.

G. SUMMARY

Mescaline and the four mescaline-like amphetamine derivatives
were found to produce a high amplitude, slow wave hypersynchronous
bursting activity in the electrogram from the brain of the conscious
and unrestrained cat. In an attempt to quantify this response by
electronically filtering and integrating the alternating wave forms
into a direct current signal which could be objectively measured an
8 channel frequency analyzer was constructed.

Because of variations in the results sharp quantitative dis-
tinction could not be made but the data supported the following con-
clusions: TMA is the most potent compound with MDA a close second.
DMA and mescaline have similar potencies which are 7 to 10 times less
than TMA. MMDA is intermediate between MDA and DMA. Amphetamine did
not produce hypersynchronous bursting in the brain of the cat and is
apparently acting on different central mechanisms. There is a gross
correlation between the ability of these compounds to produce hyper-
synchronous bursting activity in the brain of the cat and their
hallucinogenic potency in man.

CHAPTER V

GENERAL SUMMARY, DISCUSSION AND CONCLUSIONS

AND SUGGESTIONS FOR FUTURE RESEARCH

A. GENERAL SUMMARY OF RESULTS

The main objective of all the investigations which have been
presented in this thesis was to discover, if possible, a method, or
methods, employing experimental animals in which the central nervous
system activity of a series of compounds having closely similar chem-
ical structure could be quantitatively analyzed so that the results
would indicate some degree of empirical correlation to the hallucino-
genic properties of these agents in man. Three experimental proce-
dures were employed in this search; the results of one failed to show
any correlation; a second indicated a partial relationship; and the
third successfully ranked the compounds in approximately the same
order as they would be ranked for their hallucinogenic potency in
man.

Studies on the transcallosal evoked potential in the brain of
the anesthetized cat failed to show any positive correlation to the
human experience. Mescaline proved to be the most effective compound
for reducing the height of the evoked potential with DMA a close
second; both TMA and MDA were significantly less active. This is
almost an inverse correlation to the situation in man where TMA and
MDA are distinctly more potent than either DMA or mescaline.

The ability of the test compounds to disrupt a conditioned
avoidance response in mice had some relationship to hallucinogenic

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potency in man except in the case of amphotamine. This compound was
shown to be quite active in blocking conditioned behavior in mice and
yet it is not properly classified as a hallucinogenic agent in man.

The final test in this series of experiments was the quantifi-
cation of a distinctive electrical wave form elicited by the test
compounds in the central nervous system of unanesthetized and essen-
tially unrestrained cats. The closest empirical relationships to the
hallucinogenic potency of the test compounds in man were found em-
ploying this procedure. The results were more variable than would
have been desired but it was possible to rank the drugs in a fashion
analogous to their order of human effect. TMA and MDA were judged to
be the most active while DMA and mescaline were found to be distinct-
ly less effective. MMDA exhibited an intermediate effect and amphet-
amine itself failed to produce the characteristic electrographic pat-
tern associated with the other compounds.

B. GENERAL DISCUSSION OF QUANTITATIVE COMPARISONS OF DRUG POTENCY

One general difficulty was encountered throughout all experi-
mental investigations into the relative activity of this series of
compounds. The number of drugs involved (5 to 6 compounds) was fair-
ly high. This fact necessitated performance of comparatively large
numbers of experiments in order to obtain sufficient data for quanti-
tative comparison. In such an investigation each drug in the series
may be compared to an arbitrarily chosen standard. This can be ac-
complished either by measuring some effect produced by equal doses of
the standard and the compound being compared to it or by equating

effects of the standard and unknown and comparing the doses required
to produce them. In either case, each of the compounds would prob-
ably be analyzed at several dose levels. The nature of the experi-
mental procedures used in these particular investigations made it
difficult to handle more than one compound, and usually more than one
dose of that compound, during the course of any single experiment.
This made it necessary either to use a large number of animals or to
make repeated observations on the same subjects.

In the investigations into synaptic transmission within the
CNS the acute preparations employed could serve for only one experi-
ment and a relatively large number of animals should have been used.
Due to time limitations resulting from the fact that the work was
conducted in a laboratory other than our own the number of cats em-
ployed was somewhat small for making meaningful quantitative compar-
isons among the compounds.

The experiments involving conditioned avoidance behavior in
mice and frequency analysis of the spontaneous electrical activity in
the brains of cats relied on making repeated observations on small
groups of animals. This procedure presents the oft-encountered prob-
lem of individual variations in drug sensitivity. The fact that six
compounds were involved, all of which were administered to each ex-
perimental animal, raises the possibility of differential sensitivity
to the drugs occurring within the individual as well as the differ-
ences which might be expected to exist between subjects.

A possible solution to the problem of the necessity for per-
forming large numbers of individual experiments, when comparing

activities of a relatively long series of compounds, would be to devise procedures in which all, or most of the drugs, could be analyzed during the course of any one experiment on the same animal preparation. Such an approach did not seem feasible in our case when the particular goal we were attempting to achieve is considered. We wished to compare the activity of the test compounds in experimental animals to their potency for producing hallucinations in humans. When certain of these compounds are given in sufficient dosage to produce hallucinatory phenomena in man the effects are relatively intense and long lasting. It therefore seemed reasonable that definite, intense reactions should be sought in the animal and one could assume that such an effect would have a time course long enough to make a sequential drug analysis quite difficult. The possibilities of either tachyphylactic inhibition or additive facilitation would have to be considered in any such scheme and these contingencies coupled with a desire to work with definite and measurable changes precluded attempts at multiple drug analysis during single experiments.

C. SUGGESTIONS FOR FUTURE RESEARCH

Of the three types of experiments performed during the course of this research the study of spontaneous electrical activity in the central nervous system apparently offers the most promise for establishing empirical relationships between the activity of this particular series of amphetamine derivatives in animals and their hallucinogenic potency in man. There are a number of obvious experiments which could be done in order to determine if this method might have

application to psychoactive compounds in general. The effects of other psychomimetic drugs, such as LSD-25, bufotenine, psilocybin and cyclohexamine, which have dissimilar chemical structures to our test compounds, should be analyzed. Various ataractic agents, such as chlorpromazine, reserpine, and azacyclonal, could be tested for their ability to inhibit the observed changes in the cerebral electrogram. Such experiments are contemplated.

Another approach to the general problem of finding animal models to gain correlative information on the activity of centrally active compounds in man stems from R. G. Heath and his collaborators; their work has been previously mentioned in this chapter. While these investigators have touched on many physiological aspects of the schizophrenic process we would like to review briefly only a few facets of their work which pertains to the problem of using a sub-human species to study substances which are psychotogenic in man. Heath and co-workers have demonstrated, over the past 12 years or so, that in at least 40 schizophrenic patients an abnormal electrical pattern consisting of slow wave and spiking phenomena could be demonstrated in the septal, amygdaloid and hippocampal areas of their brains. These deviant electrical patterns became exaggerated during periods of active psychotic behavior and were attenuated while the schizophrenic process was in remission. Eight control, or non-psychotic, subjects did not demonstrate these electrical abnormalities (Heath, Leach and de Balbian, 1962). Their laboratories have also reported the isolation of a protein fraction from the blood serum of schizophrenic patients which when injected into "normal"

volunteers produces abnormal "schizophrenic-like" behavioral responses. This protein fraction, which they named "taraxein," will also produce an abnormal electrical pattern in the septal and hippocampal regions in the brain of the monkey and this electrical response is reported to be very similar to that seen during endogenous psychosis in patients. The monkey preparation is used routinely by Heath as a bio-assay for the protein "taraxein" (Heath, 1957). As previously discussed in this chapter, both LSD and mescaline were found to produce the same paroxysmal, hypersynchronous activity in the brains of both the monkey and human schizophrenics with chronically implanted electrodes (Monroe and Heath, 1961; Monroe, Heath, Mickle and Llewellyn, 1957).

The above observation that psychotogenic substances are capable of producing hypersynchronous bursting activity in specific areas of the central nervous system of monkeys, which is in many respects similar to electrical phenomena from comparable sites in the brains of psychotic patients, coupled with our own observations of drug-induced hypersynchronous activity in the brain of the cat, lends support to the hope that suitable animal preparations could be devised for quantitatively assaying substances which influence abnormal or psychotic behavioral patterns in the human. We believe that such a tool would be a useful addition to the growing field of biological psychiatry.

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